Food·Drug·Cosmetic Law

Journal

The editorial policy of this JOURNAL is to record the progress of the law in the field of food, drugs and cosmetics, and to provide a constructive discussion of it, according to the highest professional standards. The FOOD DRUG COSMETIC LAW JOURNAL is the only forum for current discussion of such law and it renders an important public service, for it is an invaluable means (1) to create a better knowledge and understanding of food, drug and cosmetic law, (2) to promote its due operation and development and thus (3) to effectuate its great remedial purposes. In short: While this law receives normal legal, administrative and judicial consideration, there remains a basic need for its appropriate study as a fundamental law of the land; the JOURNAL is designed to satisfy that need.

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About the Authors

This October JOURNAL carries the text of a lecture which is being delivered this month and next by Charles Wesley Dunn in Sweden, Denmark, West Germany and England. Mr. Dunn is discussing "The Food and Drug Law in the United States" and the Federal Food, Drug, and Cosmetic Act, in particular, in the two first-mentioned countries by government invitation. Lectures at the Universities of Bonn, Heidelberg, Hamburg and Munich are to follow and, finally, he will address a joint meeting of the British Association of Public Analysts and of the Food Group of the British Society of Chemical Industry, in London.

In his lecture, Mr. Dunn, who certainly needs no introduction to JOURNAL readers, notes that he discusses this law as president of The Food Law Institute.

Ever since the symposium article "Procedures for the Appraisal of the Toxicity of Chemicals in Foods" appeared in the September, 1949 issue of this magazine, expressions of interest in the subject and the symposium, as well as requests for copies of the issue, have reflected the vital importance of that study, as made by Arnold J. Lehman, M. D., Edwin P. Laug, Geoffrey Woodard, John H. Draize, O. Garth Fitzhugh and Arthur A. Nelson, M. D. Recently, Dr. Lehman and his coauthors, together with half a dozen other Food and Drug Administration experts, undertook to revise the article and bring it up to date. The excellent revision, as it appears in this issue, not only notes recent developments and new techniques in appraisal procedures, but covers the subjects of drugs and cosmetics, in addition to food. In submitting the completed study, Dr. Lehman wrote:

"The preparation of the manuscript has been quite an undertaking and I hope it will be received with as much enthusiasm as the authors exhibited in writing it."

Arnold J. Lehman, M. D., is Chief of the Division of Pharmacology of the Food and Drug Administration. Before joining FDA in 1946, he served on the instructional staff in pharmacology of several medical schools, and was professor of pharmacology and departmental head of the University of North Carolina School of Medicine.

Wilbur I. Patterson spent 11 years with the Administration's Division of Food. During 1954, he was put in charge of the Biochemical Unit of the Dairy Products Section, Eastern Utilization Research Branch, United States Department of Agriculture.

Bernard Davidow came to FDA in 1946, and is now Chief of the Acute Toxicity Branch, Division of Pharmacology. He is a specialist in biological assay techniques and chemical analytical methods.

As pharmacologist in the Division of Pharmacology, **Ernest C. Hagan** has been active in studying the acute toxic effects of additives. He engaged in work in the physics laboratory of the National Bureau of Standards before joining the Administration in 1944.

Geoffrey Woodard entered the Division of Pharmacology in 1936. He is Chief of the Pharmacodynamics Branch of the division.

Prior to joining FDA in 1935, **Edwin P. Laug** was active in nutritional research at the University of Tennessee and the University of Pennsylvania. He is Chief of the Physiochemistry Branch of the Division of Pharmacology.

John P. Frawley had spent four years with the Administration before he left it in 1952 to serve as Assistant Chief of the Biochemistry Department at Army Medical Service Graduate School and laboratory officer for the Surgical Research Team in Korea. On his return to FDA, he was appointed Assistant Chief of the Chronic Toxicity Branch, Division of Pharmacology.

O. Garth Fitzhugh joined the Administration in 1939 as a pharmacologist. He is now Chief of the Chronic Toxicity Branch.

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Food·Drug·Cosmetic Law Journal

The Food and Drug Law in the United States

By CHARLES WESLEY DUNN

This Lecture Is Being Given During October and November, 1955, in Sweden and Denmark, by Government Invitation; at the Universities of Bonn, Heidelberg, Hamburg and Munich, in West Germany; and at a Joint Meeting of the British Association of Public Analysts and the Food Group of the British Society of Chemical Industry, in London

Introduction

"On this distinguished occasion I am honored to discuss the food and drug law in the United States, first as a whole and then with respect to its supreme Federal Food, Drug, and Cosmetic Act of 1938. This law requires international review, because the federal part regulates the importation and exportation of its products; aside from our national tariff legislation, which is another matter in both policy and application. And it also invites such review for comparative international study, because this law has a profound social and economic and legal importance later demonstrated.

"I will discuss this law as president of The Food Law Institute in New York; I will do so as a lawyer (not a scientist) and to publish its discussion further in our country. The Institute is a pioneer and unique public organization established by leading food manufacturers (named in *Annex A*), as a contribution to the national welfare. It is designed to educationally provide necessary knowledge about our food and drug law and also that in other countries, because of its great public and industrial significance; and to do this through authoritative university (especially law school and also public health school) in-

struction in it and published research studies of it and informative public discussions on it and by other appropriate means. One is the publication of the FOOD-DRUG-COSMETIC LAW JOURNAL, which is the organ of its law and one having both a national and an international circulation at the highest levels.

"The Institute has made large progress in implementing that educational objective; and its public trustees include the foremost officials on its law in the United States and Canada and also the deans of major university law schools throughout our country, which are now widely teaching this law on both a graduate or an undergraduate basis. Its university program mainly includes a national center of graduate instruction in this law under my professorial direction, at the New York University School of Law; which has approximately fifty lawyer students or more each year, who include five national fellows thus trained to become legal experts in such law for government or university or private service across the land. In short: the Institute has thus opened a new and invaluable educational world for the food and drug law; it is dedicated to the proposition that the truth about this essential law is found in due knowledge of it; and that such truth is required for the welfare of our people.

Part I: The Law As A Whole

"*(a) Its Organic Nature.*—The food and drug law in the United States is manifestly one to regulate commerce in its products; and also their professional use, principally in the drug area. It has two remedial purposes, broadly speaking. The first and public purpose is to protect the public health, by insuring the safety and purity and integrity of its vital food and drugs. The second and economic purpose is to provide fair trade in these products, by requiring their true and honest and informative sale.

"This law is principally divided into two parts, from a jurisdictional standpoint. The first part is a federal law enacted by Congress, to regulate interstate (or national) and foreign (or international) commerce; and it chiefly governs manufacturers.[1] The second part is a state (including the local municipal) law[2] enacted in the forty-

[1] Congress also regulates commerce in the District of Columbia and the territories and possessions of the United States. The self-governing territories (principally Alaska, Hawaii and Puerto Rico) have enacted important food and drug laws.

[2] The municipal food and drug laws are significant in large cities, first including New York.

eight states, to regulate intrastate commerce; and it chiefly governs retailers. This jurisdictional division is required by our dual form of government, federal and state; but it is subject to weighty qualifying exceptions. For Congress may use its power over interstate commerce to largely or generally regulate intrastate commerce, in addition; Congress may also use its general tax power to regulate all domestic commerce; and Congress is further authorized to fix the standard of weights and measures for such commerce. And we shall see that all of these exceptions have penetrated our food and drug law.

"Moreover the law before us is practically divided into two parts, from a regulatory standpoint. The first part is a basic law, prohibiting defined harmful adulteration and misbranding and false advertisement of all food and drugs; in order to prevent the consumption of any that may kill or injure and the purchase of any that may deceive or defraud. It is predominantly a federal although importantly a state law; its adulteration and misbranding concept is historically old; but its false advertisement concept developed in the twentieth century, although it was needed before then. Whereas the second part is a supplemental law for the additional regulation of food and drugs, on the basis of particular articles and subjects. It is largely a state although importantly a federal law; it is a multitude of different statutes; and we shall see that its regulation may rise to the point of completely outlawing a dangerous or fraudulent product. Taken as a whole this supplemental law has a vast corpus, little surpassed in our entire commercial law. For it includes a tremendous and ever increasing number of legislative enactments and official pronouncements and administrative rules or regulations and legal opinions and judicial decisions. They could fill many and progressively expanding volumes; and The Food Law Institute is undertaking the enormous task of systematically publishing them, in an organized form.

"But on the other hand this law is inherently an undivided one. For it generally expresses the legislative philosophy of our free institutions, as controlled by our written federal and state constitutions. It is the philosophy of a government under law and not by man. A law that must be authorized, reasonable and just, in its substance and procedure; which the courts may invalidate, if it does not meet any of these fundamental tests. And a law that applied to private business requires its competitive conduct and only additionally regulates it to the extent demanded by the public safety and welfare.

Whereby each member of such business is otherwise left free to achieve the success earned by his own efficiency and public service. This is the order of free business enterprise under law and constitutional safeguards, which has made our country economically great and socially progressive.

"*(b) Its Historical Development.*—Before explaining the basic and supplemental parts of the food and drug law in the United States, I should pause to make two background observations on it for perspective. The first observation is that this law is historically derived from England, where its parent law is rooted in the common law of the people gradually built by them for their daily well-being. We find a brief reference to it in the Magna Carta of 1215, which established one measure of wine, ale and corn throughout the realm. But it actually goes far back of that date, to the most ancient times; and it eventually developed from a mixture of general and ecclesiastical and trade and professional rules, into a government public law. Consequently we in our country are happy to learn that Parliament is now in the course of modernizing and strengthening this foundation law of the United Kingdom; and that the other nations of the British Commonwealth have so notably developed it, especially Canada which has become a world leader in it.

"The English settlers brought that parent food and drug law to their North American colonies, where it began in a crude pioneer form.[3] It then necessarily evolved from a colonial into a state law, when our country became an independent nation of united states; and it thereafter naturally evolved into a federal law in addition, as our national government perforce assumed its constitutional role of regulating interstate and foreign commerce to promote the general welfare. In this evolutionary process our food and drug law emerged from its original English pattern into a distinctive national plan of its own, drawn to fit the characteristic political institutions and economic conditions and social needs of our country. And in such process the state law was dominant before the twentieth century, although important federal legislation was enacted then. But in that century the federal law became dominant in a major sense, with important state legislation complementing it.

"Hence our food and drug law is a very old one, which started when our country was first settled and is nearly our oldest commercial

[3] The food and drug law established by the French and Spanish settlers did not significantly affect the historical development of this law in the United States.

law. Moreover its regulatory evolution accurately portrays the advancing changes in our commercial and personal food and drug standards of living, down the years of time. This is necessarily so. For the laws of a democracy, made by the people to regulate their daily life, are always a true mirror of their economic and social progress.

"*(c) Its Modern Rise.*—The second background observation is that the food and drug law in the United States began its modern rise in approximately the last half of the nineteenth century, or somewhat more than one hundred years ago; and which then reached its climax in the first half of the twentieth century. This initial rise was basically caused by the tremendous industrial revolution in our country, which mainly got under way in that earlier period.

"For it was the period when industry was practically organized on a corporate basis; when epochal scientific inventions and natural discoveries created great new industries; and when old industries greatly expanded, through their economic progress and technological improvement. It was also the period when essential manufacturing industries vastly grew, to satisfy the consumption needs bred by a huge increase in population and its urban concentration. And it was further the period when broad transportation and communication systems were established, which permitted infinite interstate and foreign commerce. In short: that was the period when our country progressed from a small local economy into a large national and international one as well; when its industrial genius was released, for maximum exercise in a dynamic order of free business enterprise; and when it began an historic march since continued, to provide our people with the highest attainable standards of living. The food and drug industries necessarily joined in this march, because of their vital need and enhanced value. And they went on to have an historic renaissance of their own in approximately the first half of the twentieth century, which is later explained.

"But while this industrial revolution in that earlier period conferred fundamental economic and social benefits, it was incidentally accompanied by a serious public danger and evil. The danger of a wrongful private monopoly of commerce, subversive of our free institutions; and the evil of harmful commercial practices, detrimental to our national welfare. This situation arose from a condition of unrestrained and ruthless competition in a pioneer industrial era; it clearly required correction by public laws, federal and state; and the

principal correction needed was a federal one, because such danger and evil were primarily at a national (aside from an international) level. Therefore Congress eventually began to enact a series of major laws to protectively regulate interstate and foreign commerce, which have since grown apace. The first three laws should be mentioned, because of their legislative rank and the direct relation of one to our discussion. The first law was the 1887 Interstate Commerce Act, to prevent discriminatory transportation rebates in such commerce. The second law was the 1890 Sherman Antitrust Act, to outlaw any private monopoly from such commerce; which was later supplemented by legislation to further outlaw all unfair competitive methods from such commerce, which are the seed of that monopoly. The third law was the original 1906 Federal Food and Drugs Act, to prohibit the adulteration and misbranding of its most essential products in such commerce; which was succeeded by the existing Federal Food, Drug, and Cosmetic Act of 1938. Hence our first basic national food and drug law has the historic significance of being the third major law enacted by Congress, to regulate interstate and foreign commerce at all. And while this law did not reach the statute books until early in the twentieth century, it was preceded by broad state and limited federal legislation in its area increasingly enacted in approximately the last half of the nineteenth century. That is why the former legislation was dominant in such earlier period.

"*(d) Its Basic Law.*—We will now return to the basic part of the food and drug law in the United States. It is jurisdictionally divided into a federal and a state law. The federal law is again divided into two parts, from a regulatory standpoint. The first and major part is the 1938 Federal Food, Drug, and Cosmetic Act. It prohibits defined harmful adulteration and misbranding of its products, in interstate and foreign commerce; and it is administered by a Food and Drug Administration later described. Whereas the second and complementary part of this law is the 1938 amendment of the Federal Trade Commission Act. For it prohibits defined harmful false advertisement of these products, in such commerce; and it is separately administered by the Commission. That Act also broadly prohibits any unfair competitive practice, in such commerce; whereby it may further reach the adulteration and misbranding of these products. Conversely the Federal Food, Drug, and Cosmetic Act also reaches any false advertisement of these products, which accompanies them; and it may determine their labeling misbranding, from the standpoint of their general advertisement.

"Hence the federal basic law is thus an overlapping one in application and a divided one in administration, with the result that there may be (as there has been) a conflict in its dual operation. That is a fundamentally unsound condition of this law, which may be ameliorated but not resolved by coordinating administrative agreement between these two agencies. For such a basic national law against the harmful adulteration and misbranding and false advertisement of the same vital products is inherently a united one, in objective and policy and operation. Therefore it should be consolidated in the Federal Food, Drug, and Cosmetic Act, because it is the dominating part of this law; and also for the reason that the Food and Drug Administration was specially organized and is best equipped to administer such law, as hereinafter explained. Moreover this Act was originally drafted to contain that false advertisement law; and its addition was a main reason for proposing this Act, to replace the original 1906 Federal Food and Drugs Act which did not prohibit a false advertisement of its products. But the Federal Trade Commission persuaded Congress to transfer that law to its own Act, on the ground that it is a national law against all false commercial advertisement; which is literally so. Moreover the fact is that this Commission is mainly a legal and economic agency, principally organized and equipped to administer its major supplemental antitrust law; that it does not possess the scientific and technical facilities to administer a food and drug law, for the protection of public health; and that it must rely on those of the Food and Drug Administration, in any event. But I should add here the following advisory statement: that false advertisement law should be consolidated in the Federal Food, Drug, and Cosmetic Act, only to the extent it deals with matters which are regulated by this Act; and its enforcement should include a cease and desist administrative procedure as now, adapted to the operating needs of such a law; which may be replaced where necessary by appropriate court proceedings against flagrant violations.

"As to the state (including the municipal) basic law: it is analogous legislation enacted in the forty-eight states. This legislation likewise prohibits a defined harmful adulteration and misbranding and false advertisement of the same products, but here in intrastate commerce; and it is administered by varied local agencies. This law significantly differs from the federal basic one, in the following (among other) respects: it does not contain legislation similar to the Federal Trade Commission Act; in numerous states it consolidates its adultera-

tion and misbranding and false advertisement prohibitions; in fifteen states it prohibits the general advertisement of drugs for listed serious diseases (see *Annex B*), subject to administrative and professional exceptions; and it more or less contains additional or different product requirements. Furthermore taken as a whole and after making the necessary exceptions this state law is administratively weaker than the federal basic one and legally subordinate to it. For the administration of the latter law is better organized and financed, comparatively speaking as a rule; the major Federal Food, Drug, and Cosmetic Act largely regulates intrastate (in addition to interstate and foreign) commerce, as we shall see; and a state law may not constitutionally interfere with an authorized federal regulation of such local commerce. This interference arises, for example, where a state law requires a conflicting standard of identity for an interstate product; or prescribes a conflicting name for it; or prohibits a required federal labeling for it; and it may have a manifold broader reach. However notwithstanding this constitutional restraint and perhaps sometimes consistent with it, the state law now varies from the Federal Food, Drug, and Cosmetic Act to a large extent, in its major definitions of product adulteration and misbranding. This variation creates a difficult problem for manufacturers of packaged food and drugs marketed in interstate (aside from foreign) commerce; and to solve it the Association of Food and Drug Officials of the United States has recommended a model state food, drug, and cosmetic bill for general state enactment, which is uniform with that Federal Act in its product regulation. But notwithstanding that such Act has now been effective for more than seventeen years, this official uniform bill has only been wholly or partly enacted in twenty-six states to date; and the other state food and drug laws are still mostly patterned on the outmoded and repealed 1906 Federal Food and Drugs Act. This astonishing situation is caused by local state conditions, beyond discussion now; and it is improbable that the states will generally enact the model uniform bill, in the foreseeable future. Hence the alternative remedy is comity of administrative action in the circumstances, between the federal and state governments; and fortunately good progress is being made in this direction, through that official Association.

"*(e) Its Supplemental Federal Law.*—The supplemental federal law is organized into a food and a drug one, from a regulatory standpoint. The food law is a series of divers statutes for the additional

regulation of important foods, except where otherwise provided. And they may be principally summarized as follows:

"*(1) Meat Inspection Act:* This Act prohibits interstate and foreign commerce in meat and meat food products, unless they have been government inspected and passed as fit for human food;[4] and it administratively controls additives in such products, including dyes and chemicals. (They are listed in the regulations under this Act, on a permitted and prohibited basis.) The Federal Food, Drug, and Cosmetic Act exempts meat and meat food products governed by the Meat Inspection Act, to the extent it regulates them. *(2) Butter Standard Act:* This Act establishes an identity standard for butter, for the purposes of the Federal Food, Drug, and Cosmetic Act; and it is required to contain not less than 80% of milk fat. *(3) Skim Milk Act:* This Act provides that such milk may be called nonfat dry milk solids or defatted milk solids, for the purposes of the Federal Food, Drug, and Cosmetic Act; and it is required to contain not over 5% of moisture and not over 1½% of fat unless otherwise indicated. *(4) Import Tea Act:* This Act prohibits the importation of teas below their government standards of purity, quality and fitness for consumption; but substandard teas (also tea waste, siftings and sweepings) may be imported to manufacture theine, caffeine or other chemical products, under administrative regulations. *(5) Import Milk Act:* This Act prohibits the importation of milk or cream except by government permit granted on compliance with defined production and composition standards, subject to exemptions. It is expressly designed both to protect the public health and to promote the dairy industry. *(6) Filled Milk Act:* This Act wholly outlaws interstate and foreign commerce in such milk, for the declared reason that it is an adulterated food injurious to the public health and also a public fraud. It is defined to principally include milk blended with non-milk fat, in imitation or semblance thereof; but certain dietary products for professional use are exempted. *(7) Filled Cheese Law:* This Law imposes a tax on such cheese, both domestic and imported, and on its manufacturers and dealers; and then regulates its production and sale. It defines filled cheese and exempts certain products not sold as cheese or as substitutes therefor. And it is a tax law to reach and suppress all commerce in that product. *(8) Adulterated and Process or Renovated Butter Law:* This Law likewise imposes a tax on such butter, both domestic and imported, and on its manufacturers and dealers; and then likewise regulates its production and sale. It defines such butter and makes no exemptions. And it is also a tax law to reach and suppress all commerce in that product. *(9) Oleomargarine Law:* This Law imposes a suppressive tax on imported oleomargarine; and then permissively regulates its domestic manufacture and sale. It defines such product and exempts certain shortening and salad dressing (etc.). It was originally designed to suppressively tax domestic colored oleomargarine, in particular; for competitive advantage to butter. But this tax was repealed for the general welfare; and that product is now practically regulated by the colored oleomargarine (or margarine) amendment of the Federal Food, Drug, and Cosmetic Act. This amendment reaches all domestic commerce in and public service of such oleomargarine; it defines that product; it prohibits any unfit for food; it requires the packaging and special labeling of that product; it also requires a prescribed notice of its service in a public eating house; and it further requires its service therein only in a

[4] The Meat Inspection Branch of the United States Department of Agriculture, which administers this Act, has promulgated regulations for an analogous inspection of food products for dogs, cats and other carnivora, upon application and its official approval.

triangular shape. In addition the false advertisement law of the Federal Trade Commission Act particularly regulates such advertisement of oleomargarine. *(10) Standard Grade Apple Law:* This Law establishes standard grades for apples packed in barrels for interstate or foreign commerce; and it regulates the branding of such barrels. *(11) Export Apple and Pear Law:* This Law prohibits the exportation of apples or pears in packages not accompanied by an official certificate of compliance with minimum government grades of quality. It is expressly designed both to promote foreign trade in these products and to prevent a misrepresentation of their quality. *(12) Standard Fruit and Vegetable Container Law:* This Law establishes standard containers for fruits and vegetables in general and particular, for all commerce in them. *(13) Agricultural Product Standards Law:* This Law authorizes the government to establish standards of quality, condition, quantity, grade and packaging for agricultural products, for uniform practices in their commerce; and to certify compliance with them, for interstate commerce. Such products are broadly defined to include all agricultural products, together with fish and shellfish, and also their processed and manufactured products. It is a law expressly designed to promote agriculture; and we should note here the supplemental law to establish United States standards of quality and condition for grains. *Addendum:* See also the specifications and tolerances and regulations established by the National Bureau of Standards of the United States Department of Commerce, for commercial weights and measures and weighing and measuring devices; which also deal with containers and devices for food.

"Whereas the supplemental federal drug law is likewise a series of divers but fewer statutes for the additional regulation of dangerous drugs, in particular. And they may be principally summarized as follows:

"*(1) Federal Narcotic Drug Law:* This Law basically applies to opium, isonipecaine, coca leaves and opiate and any manufacture of them and any chemically identical substance. It first prohibits the importation of any narcotic drug, except amounts of crude opium and coca leaves officially found necessary to provide for domestic medical and other legitimate uses; and only under administrative regulation. But no crude opium may be imported to manufacture heroin; no smoking opium may be imported; and its domestic manufacture is suppressively taxed. In addition the exportation of any narcotic drug is strictly regulated. The Law next fully controls all domestic commerce in and the professional use of narcotic drugs for medical and other legitimate uses, except preparations of limited narcotic content. It does so by taxing such drugs and all persons importing, manufacturing, selling and professionally using them; and by a system of tax stamped packages, annual registration, official order forms, specified records and periodic reports. The Law then similarly controls the commerce in and professional use of marihuana. The Law also imposes a government license control over the domestic production of opium poppy; and likewise over the domestic manufacture of opium and opium products. It further controls the domestic sale of opium poppy seeds, except for use as a spice seed or to manufacture oil. And it finally authorizes a government acquisition of opium poppy seed, for the production of opium poppy to manufacture legitimately necessary opium and its products. *(2) Human Biological Drug Law:* This Law applies to any virus, therapeutic serum, toxin, antitoxin or analogous product and to arsphenamine or its derivatives (or any other trivalent organic arsenic compound), for the treatment of man. It prohibits interstate or foreign

commerce in any such drug, unless it was produced at an establishment holding a government license; and unless its package is marked as prescribed. It authorizes a government inspection of each licensed establishment; and it requires that every licensed establishment shall comply with government standards insuring the continued safety, purity and potency of such drugs. We may add that it is the law controlling polio vaccine, which has recently been in the public mind. *(3) Animal Biological Drug Law:* This Law applies to any virus, serum, toxin or analogous product, for the treatment of domestic animals. It first prohibits interstate commerce in any such drug, which is worthless, contaminated, dangerous or harmful. It next requires that the domestic production of such drugs shall comply with official regulations and shall be in establishments holding a government license. It then authorizes the importation of such drugs only by government permit. And it finally provides for a government inspection of imported drugs and all production establishments. We should add that this law is designed to promote animal agriculture, in addition to protecting the public health; and that it is designed, for example, to control hog cholera. *Addendum:* There are other related federal laws illustrated by those regulating industrial alcohol, contraceptives, abortifacients, and the mailing of poisons. See also the specifications and tolerances and regulations established by the National Bureau of Standards of the United States Department of Commerce, for commercial weights and measures and measuring devices; which control prescription drug scales. See further the weights and measures provisions in the United States Pharmacopoeia, the National Formulary and the Homoeopathic Pharmacopoeia of the United States, which are official under the Federal Food, Drug, and Cosmetic Act.

"The foregoing summary review of the supplemental federal food and drug law discloses pregnant annotating facts. The first fact is that this law is more drastic than the federal basic one, because it involves a permissive government control of major foods and drugs. The second fact is that its drug law is more drastic than its food law, because the former controls inherently dangerous products. The third fact is that this law rises to the point of wholly outlawing a dangerous drug, heroin, and a fraudulent food, filled milk. The fourth fact is that this law uses the general tax power of Congress, to suppress all domestic commerce in three foods, filled cheese, adulterated butter and process or renovated butter; but the United States Treasury Department nevertheless reports a small current revenue from their sale. The fifth fact is that this law also uses the general tax power of Congress, to place all manufacture of and commerce in and professional use of narcotic drugs under a very strict government control; which (as indicated) mounts to outlawing heroin, by prohibiting its manufacture. The last two facts illustrate the oft-quoted dictum by former Chief Justice Marshall of the United States Supreme Court that 'the power to tax is the power to destroy'. The sixth fact is that where this law thus taxes the importation of food and drugs, it virtually becomes a tariff law as in the case of legitimate opium; except when

the tax is intended to stop their importation, as in the case of oleomargarine and smoking opium. The seventh fact is that on its dairy food side this law has been thus competitively misused in the past to suppress oleomargarine, especially where colored; and that is wrong. The eighth fact is that on its agricultural food side this law is designed to promote the agricultural economy, in addition to protecting public health and preventing commercial fraud. And the ninth fact is that on its drug side this law is designed to regulate the practice of medicine, in addition to such public and commercial remedial purposes.

"*(f) Its Supplemental State Law.*—This supplemental state food and drug law is (as previously indicated) an immense body of infinitely varied legislation, regulating intrastate commerce. Consequently it can only be outlined here on the following classified basis, which is presently sufficient.

"The food law may be principally divided into five classes. The first class is legislation to regulate establishments for the production and sale and public service of food, to insure their sanitary conduct; and it more or less involves a government license of them. These establishments include (for example) slaughterhouses, dairies, milk product manufactories, general food factories and agricultural food canneries, cold storage plants (also otherwise regulated), meat markets, bakeries, confectioneries, soda fountains and public eating houses. The second class is legislation to regulate the composition and labeling of specific foods, which should be consistent with the Federal Food, Drug, and Cosmetic Act. They mainly include agricultural food products; and they also include such miscellaneous products as sea foods, candies, liquors, wines and soft drinks. The third class is legislation for the nutritional enrichment of major foods. It includes *(1)* laws in twenty-seven states [5] and also in Puerto Rico and Hawaii to enrich flour and white bread (together with rolls and buns), as provided (with minor differences) by the standards for these enriched foods under the Federal Food, Drug, and Cosmetic Act (later explained); *(2)* laws in five southern states [6] to enrich corn meals and corn grits, as provided by the standards for these enriched foods under that Act (later explained); *(3)* laws in seven states [7] to enrich oleo-

[5] They are: Alabama, Arkansas, Colorado, Georgia, Indiana, Kansas, Kentucky, Louisiana, Maine, Massachusetts, Mississippi, Nebraska, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Rhode Island, South Carolina, South Dakota, Texas, Washington, West Virginia and Wyoming.

[6] They are: Alabama, Georgia, Mississippi, North Carolina and South Carolina.

[7] They are: Alabama, Iowa, Louisiana, Mississippi, South Carolina, Tennessee and Texas.

margarine, governed by the standard for this food under that Act (later explained); and *(4)* a law in Puerto Rico to enrich rice (explained in *Annex C*). A similar law was proposed but not enacted in the Philippine Islands. No standard for enriched rice has been established under the Federal Act. But one state (South Carolina) has considered and not yet enacted it. We should note however that about 8 to 10% of domestic rice is exported to Puerto Rico, which must comply with its enrichment law. It should be added that the United States Food and Drug Administration is constantly advising rice millers for the enrichment of their foods. And it should be further added that there is no state legislation to enrich milk. The fourth class of this supplemental food law is legislation to establish standard weights and measures for agricultural food products especially. And the fifth class is legislation to regulate animal and poultry food. In addition there is other supplemental state legislation that directly or indirectly relates to food, which need not be mentioned here.

"Whereas the supplemental drug law is principally divided into six classes. The first class is likewise legislation to insure the sanitary conduct of drug establishments. The second class is legislation to insure the safe production and sale and prescription compounding of drugs, by placing them under the supervision of officially registered pharmacists; except in the case of harmless family remedies. The third class is legislation to further insure the safe retail dispensing of dangerous drugs, by limiting it to a medical prescription basis. They include (for example) poisonous, hypnotic, biological, antibiotic, hormone and venereal disease drugs; indeed all drugs required to bear a prescription restricted label under the Federal Food, Drug, and Cosmetic Act. Other miscellaneous articles in this prescription category are hypodermic needles and syringes, abortifacients and contraceptives. The fourth class is legislation to insure the product and labeling integrity of drugs, especially official ones; which again should be consistent with the Federal Food, Drug, and Cosmetic Act, with due allowance for the special pharmacist labeling of prescription drugs. It should be generally added here that uniform state laws have been notably achieved in the narcotic drug area. The fifth class is legislation to otherwise control the marketing of human drugs, with respect (for example) to samples, auctions, vending machines and prescription drug substitution. And the sixth class includes legislation to regulate animal and poultry medicines. I should go on to say that there is other state legislation relating to drugs, such as that

dealing with industrial alcohol, medicinal liquors and wines, and disinfectants.

"The foregoing summary review of the supplemental state food and drug law likewise discloses pregnant annotating facts. The first fact is that this law is also more drastic than the federal basic one, because it significantly involves a permissive government or medical control. The second fact is that its drug law is likewise more drastic than its food one, because the former similarly controls inherently dangerous products. The third fact is that this law is importantly a sanitation one. The fourth fact is that on its food side this law is a fundamental nutrition one. The fifth fact is that on its agricultural food side this law is similarly designed to promote the agricultural economy, in addition to protecting public health and preventing commercial fraud. The sixth fact is that on its drug side this law is mainly contained in the state pharmacy laws, which are historically the major origin and practically the chief instrument of it. For it is these laws which place the production and sale and prescription compounding of drugs under the supervision of officially registered pharmacists; which basically limit the retail dispensing of dangerous drugs to a medical prescription basis; which primarily safeguard the product and labeling integrity of drugs; and which otherwise regulate them in manifold ways. Such laws are administered by state boards of pharmacy, which virtually dominate the state drug laws and sometimes also administer their basic part against general drug adulteration and misbranding and false advertisement. But it should be added that these laws may not constitutionally require the pharmacist ownership of a drug store, as they have sought to do; and that they should not be competitively misused to vest a trade monopoly of safe household medicines in retail druggists, as they have also sought to do with varying constitutional success. And it should be noted here that the state dairy laws have been similarly misused to prevent oleomargarine competition. The seventh fact of this supplemental state law is that on its drug side it is importantly effective to regulate the practice of medicine, especially from a prescription standpoint. And the eighth fact is that on its drug side this law widely regulates animal and poultry food and medicines, to a greater extent than the federal law. But that law importantly regulates them through the basic Federal Food, Drug, and Cosmetic Act and by the complementary animal biological drug law in particular; and it authorizes broad federal action (research and applied) to eradicate animal diseases.

"(*g*) *Its Fundamental Significance.*—We now have the food and drug law in the United States before us, as a whole and in general outline. This review demonstrates that it is a great public law, which is a protective lifeline extending from the original material source of food and drugs to their final vital consumption by our people. This review likewise demonstrates that it is a law of profound economic and social importance to our country. For it fundamentally governs our two most essential manufacturing industries, of which the $66 billion food industry (at the retail level) is by far our largest one; it fundamentally regulates our two most essential articles of life, on which all our people depend for their existence and well-being; and it does both mainly for the essential purpose of protecting the public health.

"Hence it is a basic public health law, of essential value. But it is more than that. For it is also a basic fair trade law, in its industrial conception. It is further a basic agricultural law, in its application to food products in this area. For we have seen that it is importantly designed to promote the national agricultural economy; and we shall see that the Federal Food, Drug, and Cosmetic Act is effective (among other things) to establish needed standards for agricultural food products and to prescribe safe tolerances for pesticide residues on natural agricultural products. Moreover this law is still further a basic nutrition law to improve the national diet, as we have noted. And it is finally a basic pharmacy and medical law, as we have also noted and will further learn in discussing the Federal Food, Drug, and Cosmetic Act. A law on which our pharmacists and physicians rely to insure the safety and purity and efficacy of necessary drugs (always including medicines).

"In addition this law is likewise one of profound legal importance to our country, in itself and for two particular reasons elsewhere cited. The first reason is that the pioneer 1906 Federal Food and Drugs Act was historically the third major law enacted by Congress, to regulate interstate and foreign commerce at all. And the second reason is that the succeeding 1938 Federal Food, Drug, and Cosmetic Act constitutionally makes an extreme and therefore an unsurpassed exercise of the Congressional power over interstate commerce to regulate intrastate commerce as well, whereby our national government has thus assumed a very important and large control of local affairs. This notwithstanding our dual form of separate federal and state govern-

ments; and because the public welfare forced that organic change, in the modern evolutionary development of our nation.

"For all these reasons the law before us must be given a high rank in our jurisprudence and a leading place in our economic and social history. It also has the important distinction of being the commercial law of most daily concern to the largest group of our people, the consuming public. That is why it is often called 'the people's law'; or 'the consumer's law'. And I should now go on to say that our food and drug industries fully and enthusiastically approve this law, from the standpoint of its national policy; for the above public reasons and the following industrial reasons. In the first place it is the foundation law of these industries that insures their product safety and purity and integrity, whereby the consuming public may have confidence in them. And in the second place the basic national law in particular created the legal basis for the incredible historic renaissance of these industries in the first half of the twentieth century. For this law freed them from the restraint of destructive product competition; it released their creative genius, for maximum constructive exercise in our dynamic free enterprise system; and it both compelled and encouraged them to develop their vast scientific and technological abilities and resources. As a result they have made greater progress in the key sciences of nutrition and chemotherapy and in the related key field of food and drug technology during the past fifty years, *than in all time before.* A progress strikingly illustrated by the essential nutritional enrichment of needed foods and by the infinite essential creation of 'miracle drugs'; none of which was previously known. And we can emphasize the latter situation by adding that almost half of the most valuable drugs today were unknown as recently as fifteen years ago. Indeed it is not too much to say that the progress of the food and drug industries in the last fifty years is unsurpassed in our national industrial world, from the standpoint of fundamental personal benefit to our people; and that is a well deserved tribute to them.

Part II: The Federal Food, Drug, and Cosmetic Act, in Particular

"(*a*) *Its General Introduction.*—I will finally discuss the Federal Food, Drug, and Cosmetic Act, in particular. For it is the supreme basic part of the whole food and drug law in the United States; and also the necessary basic keystone to it. While this Act has a broader product application (later explained), it is principally our major national food and drug law. And (taken with its preceding 1906 Act)

it has inspired the enactment of similar federal legislation, to protect other significant products. Which includes the Federal Insecticide, Fungicide, and Rodenticide Act, the Federal Caustic Poison Act, the Federal Seed Act and the Federal Wool Products Labeling Act.

"Moreover the Act before us has a special importance on this distinguished occasion, because it is our national law that mainly governs the importation and exportation of food and drugs, exclusive of the tariff law which (as indicated) is generally another matter. From the standpoint of its application to imports I need only observe that they are subject to all the requirements of this Act; that it enacts an exceptional procedure for imports; that it has similar procedural regulations dealing with them; and that the United States Food and Drug Administration has published a comprehensive guide for importers under this Act, which may be secured on request. Whereas from the standpoint of its application to exports the Act exempts them as follows, in effect: a product subject to this Act and intended for export shall not be deemed adulterated or misbranded thereunder, if (*1*) it accords to the specifications of the foreign purchaser, (*2*) it is not in conflict with the laws of the intended export country, and (*3*) it is labeled on the outside of its shipping package to show that it is intended for export. (This exemption ceases, however, if and when such product is instead offered for sale in domestic commerce.) But I now record an important exception to this export exemption. It is that export drugs must comply with the 'new drug' law of this Act, which requires that the United States Food and Drug Administration shall approve the product and labeling safety of a drug that may be dangerous to use, before it enters either interstate or foreign commerce.

"(*b*) *Its Historical Background*.—The Federal Food, Drug, and Cosmetic Act became a law on June 25, 1938, or more than seventeen years ago. It was sponsored by the late Senator Royal S. Copeland of New York; Congress spent over six years in enacting it; and (as previously indicated) it succeeded the original Federal Food and Drugs Act of June 30, 1906, to modernize and strengthen our basic national food and drug law. The founder of that pioneer Act was Dr. Harvey W. Wiley, chief chemist of the United States Department of Agriculture from 1883 to 1912 inclusive. For he made an authoritative scientific investigation of harmful food and drug adulteration and misbranding, which proved the need of a federal law to prohibit them; he was the foremost advocate of this law and an invincible driving influence for its enactment; and he then became the first administrator of it, until

he resigned in 1912. Hence the 1906 Act is rightly called the 'Wiley Pure Food and Drug Law'. Dr. Wiley was a truly great American and we hope that his name will soon be permanently enshrined in the New York University's National Hall of Fame. When an eminent sculptor (Gutzon Borglum) carved a marble bust of Dr. Wiley he said that the latter had 'the greatest face since Lincoln's'. I should note here the legislative coincidence that President Theodore Roosevelt recommended the pioneer 1906 Act, whereas President Franklin D. Roosevelt recommended the succeeding 1938 Act. Another interesting coincidence is that the supplemental Federal Meat Inspection Act also reached the statute books on the same initial day, June 30, 1906.

"It follows that our supreme national food and drug law, enacted by the 1906 and 1938 Acts, will be fifty years old on June 30, 1956. And a great national meeting will be held in our national capital on June 27 next year, to commemorate this historic national event. The Food Law Institute is organizing it, in cooperation with the United States Food and Drug Administration, the Association of Food and Drug Officials of the United States and the Association of Official Agricultural Chemists; and other commemorative meetings will be held throughout 1956. All our interested official and public and scientific and professional and industrial organizations will be invited to be represented at this national meeting; and foreign nations will also be invited to have official delegates attend it. The program for this meeting will be at the highest national level; and it is designed to educationally re-emphasize the fundamental social and economic and legal importance of this great law to our people. Its plan also includes (among other things) a commemorative resolution by Congress; a commemorative postage stamp to honor Dr. Wiley; and a ceremony to appropriately recognize the past administrators of this law, together with Mrs. Harvey W. Wiley. Its three living administrators will be invited guests of honor. They are: Walter G. Campbell, Paul B. Dunbar and Charles W. Crawford. I now refer to this commemorative meeting, because of its own significant importance and for two additional reasons. The first reason is to stimulate a foreign interest to attend it. And the second reason is to thus prove what a high place our basic federal food and drug law has in our national history and life.

"But in thus explaining the golden anniversary of our supreme national food and drug law, I should record that it was long preceded by limited federal laws in its area. The first such law was enacted in 1848, to prohibit the importation of adulterated drugs; a similar law

was enacted in 1890, with respect to food; and in 1902 a law was enacted to prohibit the false branding of food in interstate commerce, with respect to its origin. In addition Congress enacted numerous laws to regulate interstate and foreign commerce, in specific foods. They are the oleomargarine law of 1886, the filled cheese law of 1896, the tea law of 1897, the mixed flour law of 1898 (since repealed), and the adulterated and process or renovated butter law of 1902. We should also mention the first narcotic law of 1887, prohibiting Chinese from importing opium. All this legislation however was but a prelude to the enactment of the basic Federal Food and Drugs Act of 1906. The first bill for this Act was introduced in 1879; and consequently 27 years were required to enact it. This delay was mainly caused by these reasons: it was new legislation of a highly controversial nature; there was then a strong public opinion inside and outside Congress that food and drug legislation was a state matter, since it essentially regulated local manufacture and sale; and during that early period Congress was just beginning to enact legislation to regulate interstate and foreign commerce at all. Hence, taken as a whole, our national food and drug law is now actually more than 107 years old. But that fact only adds luster to the fiftieth aniversary of its major part, in 1956.

"(*c*) *Its Operating Administration.*—The Federal Food, Drug, and Cosmetic Act is administered by the Secretary of the United States Department of Health, Education, and Welfare (hereinafter called the Secretary of Health), through a Food and Drug Administration commonly known as the FDA and directed by a Commissioner of Food and Drugs. Within the framework of its law and subject to formal approval by the Secretary and the legal advice of its Principal Attorney the FDA makes its own administrative policies, programs and decisions; and it also selects its own operating personnel, equipment and other facilities, except its national headquarters. Therefore it is virtually an independent agency within these official limitations, which is parallel to the United States Public Health Service in that Department.

"The national headquarters of the FDA are in Washington, D. C., with one veterinary medical laboratory exception. They are executively located in the building of that Department; but they are scientifically located in the building of the United States Department of Agriculture. This location division is unsound and an administrative handicap. The headquarters organization consists of five executive officers, thirteen operating divisions and a Food Standards Committee.

The executive officers are the Commissioner, a Deputy Commissioner, two Associate Commissioners and an Assistant to the Commissioner (for public relations). The operating divisions are five administrative and eight technical ones. The administrative divisions are on business operations, field operations, program planning, regulatory management and state cooperation. The technical divisions are on antibiotics, cosmetics (also dealing with coal-tar colors), food, medicine, microbiology, nutrition, pharmaceutical chemistry, and pharmacology. In addition the FDA has sixteen district offices in major cities, throughout the country; and also inspection stations elsewhere. They do its essential field work (including the inspection and sampling and analyses); and also its complementary import work at ports of entry, principally New York.

"The FDA is an expert and career professional agency, specially created and organized and equipped to administer our basic national food and drug law, which is a highly technical mission; and it necessarily has a non-political status. Consequently its personnel is selected under the civil service law and according to its competitive merit system; except the Commissioner, who is named by the Secretary of Health, from the FDA organization and on the basis of his high qualifications. Moreover the FDA has had long experience in administering such law, which in effect goes back to its 1906 origin. For this agency grew out of the Bureau of Chemistry in the United States Department of Agriculture, which first administered it. But what is most important is this: the FDA has ably and efficiently and justly administered such law down the many past years, within its statutory ability to do so; and it has thus created a successful record of distinguished public service, not relatively surpassed by any other agency of the Federal Government. A record mainly achieved because the FDA has been truly dedicated and devoted to its essential public responsibility. It is that of protecting every man, woman and child in the United States from death or injury caused by the consumption of a food or drug, which may kill or injure. This is a fundamental administrative responsibility; and it makes the FDA the national protector of public health in the vital area of food and drugs. But while all this is so it is strange that the FDA has not been mentioned in either the original 1906 Federal Food and Drugs Act or the succeeding 1938 Federal Food, Drug, and Cosmetic Act, as it manifestly should be. The explanation is that it is an outside executive creation, deriving its powers from annual appropriation legislation by Congress.

"We should now add that the FDA's fundamental administrative responsibility has vastly increased since the Federal Food, Drug, and Cosmetic Act was enacted in 1938, for numerous reasons. They mainly are:

"First: The regulatory jurisdiction of this Act has been immensely expanded by its amendment. As a result: the FDA must also regulate all intrastate sale of interstate food and drugs, which is an enormous task; the FDA must further regulate all local hotel and restaurant service of colored oleomargarine to prevent its substitution for butter, which is also an enormous task; the FDA must still further regulate all drug store retailing of dangerous drugs to make certain that they are restricted to a medical prescription basis, which is likewise an enormous task; and the FDA must even further regulate all domestic production of insulin and five antibiotic drugs, to certify their product and labeling safety and efficacy, which is again an enormous task.

"Second: The regulatory jurisdiction of this Act has also been immensely expanded by the large growth of our population, which has greatly increased the number of people whose health the FDA must safeguard. That growth is strikingly revealed by the following statement: in 1939 our population was about one hundred and thirty million; it then increased to over one hundred and sixty-five million in the first half of 1955; and the government estimates that it will reach two hundred and twenty million by 1975.

"Third: The regulatory jurisdiction of this Act has likewise been immensely expanded as a result of the large growth of our national economy and income. For it has greatly increased the consumption of food and drugs and the number of commercial establishments manufacturing and distributing and otherwise handling them. That growth is strikingly revealed by the following statement: in 1939 our national gross product[8] was $91 billion; it then increased to the rate of $380 billion in 1955; the government estimates that it will reach $500 billion by 1965; and the National Association of Manufacturers estimates that it may reach $700 billion by 1975. Whereas, on the other hand, the national personal income[9] was $73 billion in 1939; it then increased to the rate of approximately $300 billion in 1955; there is an authoritative estimate that it will reach $400 billion by 1965; and there is a similar estimate that it may approximate $500 billion by 1975. These figures must be translated from the standpoint of food and drugs and that may be done by this general observation: it is officially estimated that the FDA now regulates products having an annual value largely exceeding $60 billion and commercial establishments numbering approximately 100,000; these figures represent a many-fold increase since 1939; and they will surely be greatly increased in the next twenty years or more."[10]

"Fourth: The regulatory jurisdiction of this Act has further been immensely expanded as a result of the evolutionary modern change in food and drug consumption habits; and in the modern scientific and technological advance in the

[8] *National Gross Product:* Market value of the output of goods and services produced by the nation's economy, before deduction of depreciation charges and other allowances for business and institutional consumption of durable capital goods.

[9] *National Personal Income:* Current income received by persons from all sources, inclusive of transfers from government and business, but exclusive of transfers among persons.

[10] For example: the retail food business alone was only $16 billion in 1939; it now approximates $66 billion; and there is an authoritative estimate that it may reach $125 billion by 1975. In addition the retail drug business now totals $4½ billion and the retail cosmetic business now totals $1,200,000,000.

creation and marketing of food and drugs. This evolution is strikingly illustrated by the scientific development and general consumption of new precooked, enriched, frozen and baby foods; and by the scientific discovery and general use of the 'miracle drugs'. Indeed and as previously stated greater progress has been made during the past fifty years in the basic sciences of nutrition and chemotherapy and in the basic technology of food manufacture and marketing, than in all time before. And that progress is sure to infinitely continue.

"*Fifth:* In addition to all this and other expansion in the regulatory jurisdiction of the FDA its administrative responsibility has progressively become immensely more difficult and complex, because of that very modern progress in the science and technology of food and drugs. This situation is strikingly illustrated by the analytical problems confronting the FDA in evaluating the safety of chemical additives in manufactured food and of pesticide residues on natural food; in approving the product and labeling safety of intricate 'new drugs', which may be dangerous to use; and in certifying the safe and efficacious production of complicated insulin and antibiotic drugs, which may also be dangerous to use.

"The foregoing immense expansion in the FDA's administrative responsibility, since its present Act was enacted in 1938, is in addition to its original great responsibility under this Act; and it eventually raised the question whether the FDA has adequate operating resources to keep pace with it. Such resources are principally its funds and staff and equipment and other facilities; and manifestly its funds resource is the key to the others. To secure an authoritative and objective public answer to this question the Secretary of Health appointed a representative national Citizens Advisory Committee, to study and report on it. The Committee was named on January 17, 1955 and I was a member; Congress made a special $21,000 appropriation for it; and its report was submitted on July 7, 1955. This report is a pioneer one of great historical significance; and it will doubtless have a deep impact on the FDA and the future administration of its basic Act.

"The report finds that the FDA now has insufficient funds, staff and facilities to meet its essential administrative responsibility to protect the public health, under the 1938 Act. To correct that situation it generally recommends between a three and a four fold expansion of the FDA needed personnel and facilities (including their modernization), within the next five to ten years; and an increase in the annual Congressional appropriations for the FDA, enough to finance this expansion. It also generally recommends that this expansion be progressively authorized, as fast as it can be absorbed from an efficient organizational standpoint; that each increased appropriation be based on an evaluation of the FDA record in the past year and the FDA

need in the next year; and that the Secretary of Health annually appoint a small *ad hoc* citizens advisory committee, to assist in this evaluation.

"The report then specifically recommends that the next FDA appropriation for fiscal 1957 be increased from 10 to 20% over that for the current fiscal 1956. The latter regular appropriation is $5,484,000, for the FDA administration of its 1938 Act and the supplemental federal filled milk, import milk, import tea and caustic poison acts. In addition Congress recently gave the FDA a further but separate appropriation of $300,000, to administer the polio vaccine law on an inspection basis; Congress makes a further annual appropriation for legal services to administer such acts; and the FDA also receives a self-liquidating annual income from paid services to certify insulin and five antibiotic drugs and coal-tar colors and to inspect sea food, which now approximates $1 million. This recommendation that the current FDA appropriation of $5,484,000 be increased from 10 to 20% in the next fiscal year invites three significant comments. The first comment is that such increased FDA appropriation will still be little more than that for fiscal 1952, which was $5,626,000, and for fiscal 1953, which was $5,600,000; the highest amounts in the past. And it should be added here that the present FDA administrative personnel (aside from 158 employees in the self-paying certification and inspection services and the further new employees in the special polio vaccine work) totals about 900, which is less than the peak number of 944 in fiscal 1951. For these and other reasons the truth is that the FDA has been appropriation starved from the enactment of its 1938 Act; and that in last fiscal 1955 it was required to do a vastly increased administrative job with less employees and funds than in previous years. Hence the wonder is that it has done this job so remarkably well; and it only did so because of its public dedication and operating efficiency and hard overwork. The second comment is that the current $5,484,000 appropriation to administer our basic national food and drug law to protect human health is strikingly contrasted with the current appropriation of $14,325,000, to administer the supplemental Federal Meat Inspection Act; and with the current appropriation of more than $18,000,000 for the United States Department of Agriculture to eradicate agricultural pests and plant and animal diseases (etc.). The third comment is that Canada is now spending $1,284,000 to administer its Food and Drugs Act, an analogous basic national food and drug law; in a country having a population of only 15½ million people. Which

is a relatively larger amount. In short: Canada now appropriates about 8⅓ cents a person to administer its law; whereas the United States appropriates about 3⅓ cents a person to administer its similar law.

"This report by the Citizens Advisory Committee then makes three major specific recommendations, in addition to the foregoing appropriation one. The first recommendation is that the FDA be provided with its own headquarters building, in the Washington area. This to consolidate its central organization and facilities in an appropriate edifice, adequately constructed and equipped to satisfy our modern national requirements. I attach great importance to this recommendation. For the FDA is now inadequately housed on a handicapping divided basis, as we have seen; and nothing will give it greater merited dignity and prestige in our general public and official government mind than a distinguished model home of its own in the national capital. The parallel United States Public Health Service in the same Department now has such a home; and also the Federal Trade Commission, which administers the false advertisement legislation that supplements the FDA's basic law against harmful adulteration and misbranding. Moreover Canada is just completing a fine modern home for its equivalent Food and Drug Directorate, in Ottawa. It will cost over $2,500,000; it will also house the Canadian Division of Narcotic Control; and it will be dedicated early in 1956. While our country must congratulate its neighbor on this pioneer advance, we do not wish to be surpassed by it; mainly because it is so greatly needed in our own country.

"The second recommendation by this Committee is that the FDA more and better administer its 1938 Act, by constructive education from both a general and a particular standpoint; in due balance with its necessary court enforcement. Education to give our people a full understanding of their basic national food and drug law and its essential public value; and to promote an informed industrial compliance with it. I also attach great importance to this further recommendation. For such Act is best and most largely administered on an educational basis; and the fact is that it is self administered by the regulated industries through its own and official education, to an extent substantially exceeding 99%. But there will always remain fringe violations by ignorant or unbalanced or deliberate malefactors, who commit serious offenses involving public injury or economic fraud. In this grave situation education stops and enforcement begins, for which the Committee also recommends adequate appropriations by Congress. The court enforcement authorized by this Act is seizure proceedings, to

remove a harmful article from the market; injunction proceedings, to prevent any marketing of such an article; and criminal proceedings for punishing violations by penalties of fine and imprisonment, in which the government does not have to prove a violation intent and numerous jail sentences have been imposed. But this Act also justly authorizes an administrative warning against a minor violation, to thus prevent or stop it without resort to a court enforcement proceeding. And this Act further justly authorizes an administrative hearing before a criminal enforcement proceeding, to make certain that it is fairly warranted. That statement leads me to add that the FDA should be (as it normally has been) alert to prevent an unjust criminal proceeding against a legitimate manufacturer, for an inadvertent technical violation caused by an uncertain or controversial question. That comment emphasizes the essential value of an educational administration of the Act before us, in this era of pioneer fundamental advance in the science and technology of food and drug manufacture; and it invites the following additional observation. Our food and drug industries strongly endorse the FDA administrative technique of educationally solving complex technical problems under this Act in that era, by their authoritative professional discussion on a general seminar basis. This technique has been valuably used to deal with antibiotic manufacturing problems; and it could be similarly employed, for example, to deal with food additive problems of broad industrial concern.

"As to the third recommendation by this Committee: I need only say that it makes important concrete suggestions for the general improvement of the FDA's administrative organization and conduct. But they are too many and technical to consider now; and therefore you are referred to their discussion in the Committee's report, which will be provided on request. It should be noted however that the FDA has already implemented an important recommendation, to revise its 'new drug' procedure.

"*(d) Its Product Regulation.*—In conclusion I will summarize the decisive product regulation of the Federal Food, Drug, and Cosmetic Act. It is (as we have seen) a law to prohibit defined harmful adulteration and misbranding of all its products, in both interstate and foreign commerce; and one that also largely reaches intrastate commerce, as previously explained. But it contains two important special laws, which are outside its adulteration and misbranding orbit. The first is its 'new drug' law (elsewhere described) that has its own separate jurisdiction and penalty; and it is thus distinguished from its supple-

mental insulin and antibiotic drug law that is a misbranding one. The second is the emergency food law authorizing a temporary FDA permit control over the production of food (as a class and in any locality) injuriously contaminated with micro-organisms, which also has its own separate jurisdiction and penalty; but it has not yet been used. (It could apply for example to the ripe olive situation, which previously arose.) Furthermore the misbranding and adulteration law of this Act should be approached from the standpoint that it also authorizes extensive product regulation through administrative regulations, which if valid have the force of law; but they must always comply with the Federal Administrative Procedure Act. The significance of these regulations is illustrated by saying that they are used to make food standards and to list permitted coal-tar colors and to establish safe pesticide residue tolerances and to prescribe exemptions from the drug labeling requirement of adequate directions and warnings. This reference to such administrative regulations leads me to add that the Federal Food, Drug, and Cosmetic Act fundamentally differs from the analogous Canadian Act. For the regulations under the former Act are subject to a court review of their validity, pursuant to our system of a government under law and not by man; whereas the regulations under the latter Act are conclusive. And this comment in turn leads me to further add that our Act fundamentally differs from the analogous United Kingdom Act. For the former Act is federally enforced even down to a local level; whereas the latter Act is locally enforced, as I understand.

"I should now go on to say that in defining the adulteration and misbranding of its products the Federal Food, Drug, and Cosmetic Act contains important special laws that additionally regulate individual products. They include (*1*) the butter, skim milk, colored oleomargarine and prescription drug laws, previously noted. (*2*) A wrapped meats law, which regulates their compliance with the net weight labeling requirement of this Act. (*3*) A confectionery law, which declares this food adulterated if it bears or contains any alcohol or nonnutritive article or substance (e. g., a metal trinket), except harmless coloring and flavoring and resinous glaze (not in excess of 4/10ths of 1%) and natural gum and pectin. But this law does not apply against confectionery containing less than ½ of 1% by volume of alcohol derived solely from the use of flavoring extracts; or to chewing gum because it contains harmless nonnutritive masticatory substances. (*4*) A sea food law, which authorizes an FDA inspection of the pro-

duction and packing and labeling of such food (e. g., shrimp), on the basis of an approved voluntary application and the payment of self-liquidating expense fees. (5) A pesticide residue law, which regulates the establishing of safe tolerances for pesticide chemicals in or on raw agricultural commodities (principally fruits and vegetables). On April 15, 1955 the FDA published a summary of many important tolerances and exemptions for such residues of pesticide chemicals, which may be secured on request. And at this point I direct your attention to an historic paper in the forthcoming October issue of the FOOD-DRUG-COSMETIC LAW JOURNAL on procedures for the appraisal of the toxicity of chemicals in foods, drugs and cosmetics, written by Dr. Arnold J. Lehman, the eminent chief of the FDA Division of Pharmacology, and his scientific colleagues; which thus officially and newly covers the basic area of food, drug and cosmetic additives. (6) The law requiring an FDA batch certification of the product and labeling safety and efficacy of insulin and five antibiotic drugs, before they are sold; which supplements the general 'new drug' law, requiring an FDA evidential approval of the product and labeling safety of all new drugs that may be dangerous to use, before they are sold. Both of these laws normally provide for the sale of their drugs only on the basis of a prescription restricted label; and they are each subject to due product exemptions, administratively made. The five antibiotic drugs are those containing penicillin, streptomycin, chlortetracycline (aureomycin), chloramphenicol or bacitracin. But there is a sharp difference of opinion between the FDA and some manufacturers of antibiotic drugs and between the manufacturers of such drugs, on the question whether this special antibiotic law should be continued and, if so, whether it should be extended to all other antibiotic drugs constantly reaching the market. On the one hand the FDA and some manufacturers argue that this law should be continued and thus extended, because its additional production control is necessary to safeguard the public health in the circumstances; whereas on the other hand other manufacturers argue that this law should be repealed and therefore not extended, because it was temporarily enacted to meet the emergency of a new and difficult scientific production of antibiotic drugs which no longer exists as a rule; and because the safety control of all antibiotic drugs is now sufficiently provided by the broad 'new drug' law. I see no prospect that such difference of opinion will be resolved, by repealing this special antibiotic law; and in that event it will remain in the present anomalous condition of applying to some antibiotic drugs and not infinitely to

others, which are in precisely the same production situation under the Act before us. But I add that if this law is repealed Congress should concurrently make an appropriation to adequately finance the superb FDA Antibiotics Division, whose investigatory scientific need will basically continue under this Act.

"I should now make two further introductory observations on the regulatory law of the Federal Food, Drug, and Cosmetic Act. The first observation is that this law has a broad product reach. For it applies to all food (including chewing gum) and all drinks and all drugs (including medicines) for use by man or domestic animals, together with all components of them. It also applies to all articles (other than food) intended to affect the structure or any function of the human or animal body (such as fat reducers), together with all components of them, which are here classed as drugs. It further applies to all mechanical devices for the therapeutic treatment of man or domestic animals or to affect the structure or any function of their bodies, together with all components and parts and accessories of such devices, which are defined to be adulterated and misbranded as in the case of drugs—except as provided. And it finally applies to all cosmetics for human use except soap, together with all their components. Hence this Act is far more than a food and drug law, although it is principally and basically that; its additional products were not covered by the original 1906 Federal Food and Drugs Act; and there was a precedent for its further cosmetic application. For cosmetics were previously regulated by certain state food and drug laws; and their legislative regulation goes back to ancient times, as the 1731 English law to prohibit the adulteration of hair powder indicates. The second observation is that this Act has a broad application to labeling. For it is defined to include both all labels of its products and all other printed, written or graphic matter (*1*) upon any such product or its containers and wrappers or (*2*) accompanying it.

"Having made the foregoing broad introductory statement on the product regulation law of the Federal Food, Drug, and Cosmetic Act I last come to its general definitions of adulteration and misbranding, which fix its principal food and drug application supplementing that of its special article laws.

"These definitions are very extensive and therefore I must refer you to their full statement in and under this Act, which may be secured on request. But the scope and importance of their resulting product regulation are illustrated by the following statement. The Act thus

establishes standards of identity, quality and fill of container for major foods, administratively made; and it is estimated that over 50% of all packaged foods now retailed in our country are governed by these standards. The Act thus establishes standards of purity, strength and quality for major prescription drugs in particular, scientifically made by the United States Pharmacopoeia and National Formulary and Homoeopathic Pharmacopoeia of the United States or any supplement to them; and it is estimated that these standards govern over 30% of all prescription drugs now retailed in our county. The Act thus requires the sanitary production of all food and drugs. The Act thus outlaws any food which is unwholesome and consequently unfit for consumption; and any drug which is dangerous to health, when used in the dosage or with the frequency or duration recommended in its labeling. The Act thus authorizes the administrative batch listing of coal-tar colors, which are harmless and suitable for use in food and drugs. (It should be parenthetically noted here that on December 30, 1954 the FDA announced that its Orange Nos. 1 and 2 and Red No. 32 are not harmless and suitable for use in coloring food or drugs or cosmetics for other than external application; but their use has not yet been administratively prohibited.) The Act thus outlaws any chemical or other additive in food, which is poisonous or deleterious; subject to a practically inoperative exemption. The Act outlaws any food or drug container that is wholly or partly composed of any poisonous or deleterious substance, which may render its contents injurious to health. The Act thus prohibits any deceit or fraud whatever in the sale of a food or drug, from the broad standpoint of its composition and labeling and container. The Act thus outlaws an imitation food, unless it is thus labeled as provided; and any imitation drug at all. The Act thus requires that a represented dietary food shall be labeled to disclose information about its vitamin and mineral and other dietary properties, as administratively required; and the label of such a food is thus required to define the proportion of the minimum daily requirement for each such dietary property supplied by the food when it is consumed in a specified quantity during a given period. And the Act thus further requires a needed informative labeling of all food and drugs.

The labeling of food is required to plainly indicate the following important information about it: (*1*) Its usual or common name, in case it is a non-standard food; (*2*) its official name, in case it is an official food, and also (where required) the common names of its optional ingredients; (*3*) the name and place of business of its manu-

facturer or packer or distributor, if it is a packaged food; (*4*) its net weight, measure or numerical count, subject to administrative exceptions, if it is such a food; (*5*) a declaration that it falls below its standard of quality or fill of container, where one has been administratively established;[11] (*6*)a list of its ingredients under their common or usual names, in the case of a non-standard food and where it is fabricated from two or more ingredients and subject to exceptions; (*7*) a declaration that it bears or contains an artificial flavoring or coloring or a chemical preservative, subject to administrative exceptions and (in the case of artificial coloring) to an exemption for butter and cheese and ice cream. Whereas the Act requires the labeling of a drug to plainly indicate the following important information: (*1*) its common or usual name, if it is a non-standard drug; (*2*) its official name, if it is a standard drug which must be labeled and packaged as required; (*3*) a declaration that it varies from its official standard and how, if that is the case; (*4*) the name and place of business of its manufacturer or packer or distributor, if it is a packaged drug; (*5*) its net weight or measure or numerical count, subject to administrative exceptions, if it is such a drug; (*6*) the caution 'Warning—May be habit forming', if it contains listed narcotic or other hypnotic substances together with a statement of the name and quantity or proportion of each one; (*7*) the common or usual name of each active and listed ingredient and the quantity or proportion of each one, except as administratively provided, in the case of a non-official drug fabricated from two or more ingredients; (*8*) adequate directions and warnings for its use, except as administratively provided; and (*9*) a precautionary statement that it is liable to deterioration, as provided.

"In answer to official questions submitted in advance of this lecture, I add the following comments on the above product regulation of the Act before us. The first comment is that its food standards of identity principally relate to many canned foods and vegetables; and that they also relate to the following products: flours, farina, semolina, corn meals and grits, breads together with rolls and buns, alimentary pastes (including macaroni and noodle products), milk and cream, oleomargarine, fruit butter and jelly and preserves and jams, oysters, eggs and their yolks, salad dressing, cheese and cacao. The number of these identity standards tends to constantly increase; and administrative care must be taken to see that they do not excessively

[11] This declaration is well known as a 'crepe label'."

increase and that they do not prevent scientific food progress or unjustly destroy valuable proprietary foods and that they do not descend to a food recipe level. As to food standards of quality: only two have been yet established for canned fruits and vegetables; and only four standards of container fill have been yet established, for canned fruit and vegetables and shrimp and oysters. The reason why the food standards law of this Act mainly applies to canned foods is that their packers mainly use it. The second comment is that the food identity standards now provide for the nutritional enrichment of the following major products (as explained in Annex D): flours, farina, corn meals and corn grits, bread together with rolls and buns, macaroni and noodle products, evaporated milk and oleomargarine. These standards as fortified by the analogous state ones (previously described) have basically improved the national diet; and I refer you to the guiding statement of general policy on the nutritional enrichment of food (see Annex E), jointly adopted by the Food and Nutrition Board of the National Research Council of the National Academy of Sciences and the Council on Foods and Nutrition of the American Medical Association. The third comment is that the FDA does not list the chemicals permitted and prohibited in food, which is administratively done under the Federal Meat Inspection Act; except as provided in food identity standards. Hence we must otherwise refer to related FDA administrative pronouncements or enforcement proceedings on this subject. They are illustrated by its declared opposition to these chemical additives in food (other than coal-tar colors previously referred to), pursuant to its general authority to prohibit any that are poisonous or deleterious (under which it acts to prevent or stop the use of any additive that evidentially has toxic properties): monochloracetic acid, mineral oil, nitrogen trichloride (Agene) in flours, polyoxyethylene monostearate type of chemical softeners for breads, antibiotic drugs in human food, dulcin or P-4000 as artificial sweeteners in food, and coumarin. For authoritative information on chemical additives in food I especially refer you to the following official publications, which may be secured on request: the Congressional report on this subject, made on January 3, 1951; the recent administrative paper thereon by the Food and Drug Commissioner, published in the September issue of the FOOD-DRUG-COSMETIC LAW JOURNAL; the new scientific paper thereon by Dr. Lehman of the FDA (previously noted), to be published in the October issue of this JOURNAL; and the guiding statement of principles and producers for evaluating the safety of intentional

chemical additives in food (quoted in Annex F), prepared by the Food Protection Committee and then approved by the Food and Nutrition Board of the National Research Council in our National Academy of Sciences, in November 1954. The final comment is that Congress will probably enact an amendment of the Act before us in 1956, to regulate chemical and other additives in food; in order to thus strengthen the present general law. It was recommended in the foregoing Congressional report; and there are now responsible bills in Congress, to enact it. The outlook is that the enacted amendment will be essentially one to prohibit the use of new intentional food additives until they have been adequately pretested to establish their safe use, under the conditions of their intended use, where this procedure is scientifically indicated; and to require an advance submittal of the pretesting data to the FDA and its prompt evaluation of such and other pertinent data for duly administering this Act accordingly, subject to a court review. Our food and chemical industries join the FDA in advocating such an amendment; and the only question is its form. **[The End]**

Annex A

Founder Members of The Food Law Institute

"American Can Company
Anheuser-Busch, Inc.
Beech-Nut Packing Company
California Packing Corporation
The Coca-Cola Company
Continental Baking Company
Continental Can Company
Corn Industries Research Foundation
Curtiss Candy Company
The Drackett Company
Flako Products Corporation
General Foods Corporation
General Mills, Inc.
Gerber Products Company
Kellogg Company
Kraft Foods Company
Libby-McNeill & Libby
Thomas J. Lipton, Inc.
McCormick & Company, Inc.
Morton Salt Company
National Biscuit Company
Owens-Illinois Glass Company
Pet Milk Company
Pillsbury Mills, Inc.
The Quaker Oats Company
Safeway Stores, Inc.
The Seven-Up Company
Standard Brands Incorporated
Sunkist Growers (Products Dept.)
Swift & Company
Walker's Austex Chili Company
Wesson Oil & Snowdrift Company
Wm. Wrigley Jr. Company"

Annex B

Model Uniform State Food, Drug and Cosmetic Bill Recommended by The Association of Food and Drug Officials of the United States

"The following is section 19 of this bill, which has been enacted in 15 states (with incidental changes in some), namely: Arkansas, California, Connecticut, Florida, Indiana, Iowa, Kansas, Louisiana, New Hampshire, New Mexico, North Carolina, Oklahoma, Tennessee, Virginia and Washington—

"'Sec. 19. (a) An advertisement of a food, drug, device, or cosmetic shall be deemed to be false if it is false or misleading in any particular.

"'(b) For the purpose of this Act the advertisement of a drug or device representing it to have any effect in albuminuria, appendicitis, arteriosclerosis, blood poison, bone disease, Bright's disease, cancer, carbuncles, cholecystitis, diabetes, diphtheria, dropsy, erysipelas, gallstones, heart and vascular diseases, high blood pressure, mastoiditis, measles, meningitis, mumps, nephritis, otitis media, paralysis, pneumonia, poliomyelitis (infantile paralysis), prostate gland disorders, pyelitis, scarlet fever, sexual impotence, sinus infection, smallpox, tuberculosis, tumors, typhoid, uremia, venereal disease, shall also be deemed to be false, except that no advertisement not in violation of subsection (a) shall be deemed to be false under this subsection if it is disseminated only to members of the medical, dental, or veterinary professions, or appears only in the scientific periodicals of these professions, or is disseminated only for the purpose of public-health education by persons not commercially interested, directly or indirectly, in the sale of such drugs or devices: *Provided*, That whenever the ..... * determines that an advance in medical science has made any type of self-medication safe as to any of the diseases named above, the ..... * shall by regulation authorize the advertisement of drugs having curative or therapeutic effect for such disease, subject to such conditions and restrictions as the ..... * may deem necessary in the interests of public health: *Provided*, That this subsection shall not be construed as indicating that self-medication for diseases other than those named herein is safe or efficacious.'"

Annex C

Laws of Puerto Rico Annotated (Title 24)

"Sec. 881. Nutritional qualities of rice—definitions

"(a) Rice means the grain or seed of the cereal plant Oryza sativa. (b) Whole rice means the grain or seed to which merely the skin or shell has been taken out, but which, however, substantially retains the bran, so that it contains not less than 1.35 milligrams of thiamine (Vitamin B_1), 0.3 milligrams of riboflavin, 16.0 milligrams of niacin and 13.0 milligrams of iron per pound. (c) Parboiled rice means rice which, before taking out the skin or shell therefrom, before unshelling it, has been half boiled and dried, so that even after polished

"* Insert proper designation of State Officer, Board, Department, etc."

it contains 1.2 milligrams of thiamine (Vitamin B_1), 0.15 milligrams of riboflavin, 16.0 milligrams of niacin and 13.0 milligrams of iron per pound. (d) Fortified rice means milled rice of the general appearance at present consumed in Puerto Rico, to which is added thiamine (Vitamin B_1), niacin, and iron, so that it contains not less than 2.0 milligrams of thiamine (Vitamin B_1), 16.0 milligrams of niacin and 13.0 milligrams of iron per pound; when washed in cold water, in the manner prescribed by the Secretary, it should not lose more than 15% of its content of each of these substances. (e) Secretary means the Secretary of Health of Puerto Rico. (f) Person means any natural or artificial person, business concern, corporation, association or any other entity.

"Sec. 882. No person shall store, own, sell or offer for sale, or purchase, present, grant, give, or in any manner transfer in Puerto Rico for human consumption, any rice which is not whole rice, parboiled rice, or fortified rice, as herein defined; Provided, That the provisions of this section shall not be applicable to (a) rice contained in canned foods prepared and boiled; (b) intact rice (unshelled) or to polished, unfortified white rice that may be imported for processing in Puerto Rico and then to be sold or offered for sale as whole rice, parboiled rice, or fortified rice, in which case it shall be subject to the provisions of the regulations that the Secretary may prescribe.

"Sec. 883. The Secretary shall prescribe in accordance with the provisions of law, the necessary regulations for the enforcement of sections 881-887 of this title. Said regulations shall include provisions on labeling, etc. (dealing with administrative enforcement of the act)."

Annex D

Nutritional Enrichment of Foods by Their Identity Standards Under Federal Food, Drug, and Cosmetic Act

"Enriched flour, enriched bromated flour, enriched self-rising flour:

Thiamin, riboflavin, niacin, iron
Optional: Vitamin D and calcium (except calcium required in self-rising flour)

Enriched farina:

Vitamin B-1, riboflavin, niacin, iron
Optional: Vitamin D, calcium and wheat germ

Enriched corn meals:

Thiamin, riboflavin, niacin, iron
Optional: Vitamin D, calcium, dried yeast

Enriched corn grits:

Thiamin, riboflavin, niacin, iron

Optional: Vitamin D, calcium, dried yeast

Enriched bread, enriched rolls, enriched buns:

Thiamin, riboflavin, niacin, iron

Optional: Vitamin D, calcium, wheat germ

Enriched macaroni products:

Thiamin, riboflavin, niacin, iron

Optional: Vitamin D, calcium, wheat germ

Enriched noodle products:

Thiamin, riboflavin, niacin, iron

Optional: Vitamin D, calcium, wheat germ

Evaporated milk:

Optional: disodium phosphate, sodium citrate, calcium chloride, Vitamin D

Oleomargarine:

Optional: Vitamin A (not less than 15,000 United States Pharmacopoeia units per pound); Vitamin D

"Note: These standards must be consulted for their full nutritional enrichment provisions; and they may be secured on request."

Annex E

A Statement of General Policy in Regard to the Addition of Specific Nutrients to Foods

Adopted jointly by the Food and Nutrition Board of the National Research Council of the National Academy of Sciences and the Council on Foods and Nutrition of the American Medical Association in November 1953

"During the 1930's certain nutritional deficiencies were prevalent in the population of the United States, and newly developed synthetic vitamins were being used in foods with little or no scientific guidance. In 1939, and again in 1946, the Council on Foods and Nutrition of the American Medical Association adopted its policies on the proper additions of vitamins and minerals to foods. In 1941 the Food and Nutrition Board (originally the Committee on Foods and Nutrition) of the National Research Council likewise adopted a policy on the addition of specific nutrients to foods. These statements of policy have

now been reconsidered jointly by the Food and Nutrition Board and the Council on Foods and Nutrition in the light of experience and of new developments. There is good evidence to indicate that the policies have been beneficial to the public and have encouraged sound nutritional practices. The policies are therefore reaffirmed in principle, and with revision of wording are embodied in the following statements:

"(1) With carefully defined limitations, the principle of the addition of specific nutrients to certain staple foods is endorsed for the purpose of maintaining good nutrition as well as for correcting deficiencies in the diets of the general population or of significant segments of the population. The requirements for endorsement of the addition of a particular nutrient to a particular food include (a) clear indications of probable advantage from increased intake of the nutrient, (b) assurance that the food item concerned would be an effective vehicle of distribution for the nutrient to be added, and (c) evidence that such addition would not be prejudicial to the achievement of a diet good in other respects. These requirements have been met in the specific cases indicated in paragraph (6).

"(2) The desirability of meeting the nutritional needs of the people by the use of natural foods as far as practicable is emphasized, and to that end education in the proper choice and preparation of foods and the betterment of food production, processing, storage, and distribution so as to provide more fully the essential nutrients native thereto are to be encouraged.

"(3) In order to avoid undue artificiality of food supply, foods chosen as vehicles for the distribution of additional nutrients should be, whenever practicable, those foods which have suffered loss in refining or other processing, and the nutrients added to such foods should preferably be the kinds and quantities native to the class of foods involved.

"(4) The addition of other than natural levels of nutrients to foods which are suitable vehicles of distribution may be favored when properly qualified judgment indicates that the addition will be advantageous to the public health and when other methods for effecting the desired purpose appear to be less feasible.

"(5) Whenever technologic and economic developments lead to extensive reduction in the consumption of a staple food, with a consequent nutritionally significant reduction in the intake of an essential nutrient or nutrients, consideration by qualified bodies should be given to the desirability of restoring such nutrient or nutrients to the dietary.

"(6) The endorsement of the following is reaffirmed: the enrichment of flour, bread, degerminated corn meal, and corn grits; the nutritive improvement of whole grain corn meal and of white rice; the retention or restoration of thiamine, niacin, and iron in processed food cereals; and the addition of vitamin D to milk, of vitamin A to table fats, and of iodine to table salt."

Annex F

Principles and Procedures for Evaluating the Safety of Intentional Chemical Additives in Foods

A Statement Prepared by the Food Protection Committee; Food and Nutrition Board, National Academy of Sciences—National Research Council. November, 1954

"*Foreword.*—The Liaison Panel of the Food Protection Committee, representing industry, public research and control agencies, and scientific societies, urged the Committee as part of its program for 1954 to review critically its previous statements on the evaluation of safety of chemical additives and, if it seemed advisable, to expand its statement of basic principles to include procedures for evaluating the safety of chemical additives in foods. The Committee hopes that the present statement serves this purpose.

"In its published pamphlets, 'Use of Chemical Additives in Foods' (1951), and 'Safe Use of Chemical Additives in Foods' (1952), the Committee introduced certain principles and terminology which it has since adhered to in its discussions of food chemicals. This publication does not completely replace the pamphlets mentioned, but does attempt to refine the definitions of terms and to broaden the statement of principles.

"*I. Introduction.*—This statement is intended to provide a reasonable basis for designing a program of tests for evaluating the safety of chemical additives in human foods as an aid to those concerned with the use of such additives. It is a revision of earlier statements of the Food Protection Committee.

"This report includes (a) a reconsideration and extension of definitions of terms, (b) a restatement of principles as a guide to policy decisions for use of intentional chemical additives, and (c) a suggested outline of procedures for the evaluation of safety of intentional food additives.

"*II. Definition of Terms.*—Chemical Additive: A chemical or a mixture of chemicals of known or reproducible composition used in addition to the basic foodstuff in the production, processing, or storage of a food, and present in the food as purchased. A chemical additive may be either nutritive or non-nutritive and its presence in the food may be either intentional or incidental.

"Intentional Chemical Additive: A chemical additive used in the manufacture or formulation of a food for the purpose of imparting some desired quality to the food or of serving a functional purpose in the food. In contrast, an incidental chemical additive is one present in food as a result of its having been used in the production, processing, or storage of the food but not required in the final product.

"Toxicity: The capacity of a substance to produce injury.

"Safety: The practical certainty that injury will not result from use of a substance in a proposed quantity and manner. The probability that injury will result is designated as hazard.

"*III. General Principles.*—1. A decision to use an intentional additive in foods should be based on the assurance (a) that it will be safe, and (b) that it will benefit the consumer.

"2. Results of critically designed tests of the physiologic, pharmacologic, and biochemical behavior of a proposed additive made in various species of animals can provide a basis for the evaluation of the safety of a chemical additive at a specified level of intake by man. It is impossible, however, to establish absolute assurance that the additive at this level will be completely safe for all humans under all conditions.

"3. Additives should be subject to continuing observation for possible deleterious effects under prolonged and varying conditions of use and should be reappraised whenever indicated by advances in knowledge.

"4. The safety of an additive should be appraised in terms of the minimal level of physiologic response, of the extent of its use in foods, and of the amounts that may be eaten under all likely patterns of consumption. No substance should be added to a food if there appears to be a reasonable probability that the maximum amount likely to be consumed in the human diet will produce adverse deviations from normal physiologic function.

"*IV. Basis of Evaluation.*—In order to judge the safety of the use of an additive in the light of these principles, information must be

obtained on: (A) the chemical and physical properties of the additive and, when possible, the forms to which it may be converted in the food products; (B) the biological effects of varying dosages of the additive and its conversion products, including toxicologic, metabolic, and nutritional effects; and (C) the anticipated levels and patterns of consumption.

A. Chemical and Physical Properties

"1. The additive should be identifiable in chemical and physical terms.

"2. If the additive is a mixture of chemicals, the components should be described and a reproducible composition assured by reference to physical and chemical constants.

"3. There must be available methods of adequate sensitivity and accuracy for the quantitative determination of the additive in all the foods in which its use is proposed and in animal tissues and fluids. The sensitivity required is dependent upon the toxicity of the additive. As a general guide it is currently considered satisfactory if analytical recovery is within ±10% when the additive occurs at levels of 10 ppm or more. If the additive occurs at levels around 0.1 ppm, analytical recovery within ±40% may be acceptable.

"4. Knowledge of the stability of the additive and its reactivity with the components of the foods in which it will be used is desirable.

B. Biologic Aspects

"Toxicologic aspects. In studies of toxicity, special attention should be given to: (1) uniformity of response within and among species; (2) rate, extent, and mode of detoxification and elimination; (3) tendency toward accumulation in the body; (4) occurrence of unusual or alarming reactions such as carcinogenesis; and (5) occurrence of sensitivity, tolerance, or idiosyncracy in response to the compound.

"Toxicity must be established in terms of generally accepted indices of injury such as structural, biochemical, and physiologic changes in specific organs or body systems. Toxicity testing is often guided by a knowledge of the chemical or physical properties of the substance at hand and of the effect of substances of similar properties or structure. Tests should be so designed as to emphasize any suspected potential of the substance for injury, in order to give as stringent a test as possible. Such considerations may influence the choice of a test animal in order to obtain information in the most sensitive species.

"The dosage levels investigated should range from an absence of the additive in controls through a series of intermediate levels and through at least one producing significant effects. The material should be fed at a sufficient number of levels to determine the maximum level of no response and to indicate the nature of the response at the higher levels. These observations will allow an estimate of safety in the species under study and will serve as a basis for extrapolation to the other species. In growth studies, differences may not be interpretable unless caloric intakes are equalized or otherwise taken into account.

"It is not possible to design a single program that will apply to every new additive in all its applications. As an investigation progresses, data obtained may indicate the advisability of altering the program of study as originally designed.

"With the above considerations in mind, the following tests may be suggested as a program which can be reasonably expected to yield the toxicologic data needed to assess hazard.

"1. Acute oral toxicity: The approximate lethal single oral dose should be determined in at least three species, at least one of which is a non-rodent such as the dog. This information is of value in planning studies of subacute or chronic toxicity and in the recognition of symptoms. Extension of these acute tests may occasionally be desirable.

The signs, clinical course, gross and microscopic tissue changes, and, if possible, the mode of death should be described. Surviving animals should be observed until completely recovered.

"2. Subacute oral toxicity: Results from a 90-day feeding test with ten animals of each sex at each of several feeding levels may make possible a decision as to whether the proposed use is too hazardous to warrant further toxicologic study. The information obtained may also serve as a guide in selecting feeding levels for the chronic toxicity study. The dose-response relationship should be examined.

"The data sought may include, at each of the several feeding levels, the effects on food consumption, growth, mortality rate, blood and urine composition, and organs as measured by weight and histopathologic findings. Any alterations in functions and behavior should be noted. Effects on digestibility and utilization of the ration may be important.

"The subacute feeding tests with rats may be so designed that enough rats are used at each dietary level to provide animals to be

continued in tests for chronic toxicity, in the event it becomes advisable to conduct such tests.

"3. Chronic oral toxicity: Long-term tests are conducted on the premise that the possible effects of the lifetime ingestion of an additive in food by man cannot be predicted from results of tests less stringent than lifetime feeding in a short-lived animal (approximately two years in the case of the rat) and one year or longer feeding in the dog or monkey. Obviously these tests may be either inadequate to the purpose or more exacting than necessary, but past experience has not supplied a more rational alternative.

"In the tests with rats the material is fed at selected levels in the diet to groups of ten or more weanling animals of each sex. The levels to be fed should be chosen on the basis of the data obtained in the subacute feeding tests.

"The two-year tests may include observations on: food consumption; growth; absorption, excretion, and tissue storage of the additive; mortality; organ weights; histopathologic and hematologic findings; blood and urine chemistry; such changes in behavior and function as may be determined by gross observation; and such other observations as may be indicated in special circumstances. Effects on ration digestibility and utilization and on reproduction and lactation may be especially significant.

"For dogs or monkeys, groups of three or more animals are usually fed the material under test at three or more intake levels for one year or longer. Observations are similar to those made in the chronic feeding test with rats. The dog or monkey tests are generally started after the rat studies have been in progress long enough to provide data to aid in selecting the feeding levels likely to be most informative.

"BIOCHEMICAL, METABOLIC, AND NUTRITIONAL ASPECTS. It is desirable to study in animals the biochemistry and the metabolic fate of additives. After animal tests have indicated reasonable safety, studies on man may be undertaken. Such studies may yield valuable information. Biochemical evidence can be of value in determining the safety of a compound by showing whether the additive is a product formed during the normal intermediary metabolism of foodstuff in the human body and whether it is metabolized by way of the well-known pathways of dietary components. This evidence can seldom entirely replace that from chronic toxicity tests. Special consideration must be

given the possibility that the metabolism of the additive will overload the normal pathways for metabolism of foodstuffs.

"The influence of an intentional additive upon the nutritional contribution of the foods in which it may appear must be considered. It is important to ascertain the effect of the additive on the stability of nutrients in the foods, as well as on the digestibility and utilization of the ration.

"In addition to the foregoing, two special considerations should be reemphasized: (a) The possibility of alteration of the additive after its addition to foodstuffs should be explored. The altered additive or its reaction product with an entity of the food may be more or less toxic than the original chemical. (b) The extent to which chemically or pharmacologically similar substances are present in the usual diet should be known.

C. Anticipated Levels and Patterns of Consumption

"In order to estimate the probable intake level of the additive, information on its proposed use is essential. This information includes (a) the amount of the additive technically desirable in foods, (b) the proportion of the usual diet composed of foods in which this additive may appear, and (c) the extemes of probable intakes of these foods. From this information the maximum potential consumption by individuals or special groups as well as the average potential consumption for the general population can be estimated.

"*V. Evaluation.*—One of the most difficult problems in the interpretation of toxicologic data is the translation of such data into terms of human use levels and margins of safety. Each substance presents problems peculiar to itself and requires individual consideration by those competent to exercise objective judgment of all the available evidence.

"The decision as to a safe level for an intentional food additive should be based upon such factors as the maximum dietary level that produced no unfavorable response in test animals, the severity of response in test animals at dietary levels above the no-response level, and the estimated potential for human consumption of the food or foods for which the additive is proposed."

Procedures for the Appraisal of the Toxicity of Chemicals in Foods, Drugs and Cosmetics

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This Symposium Revises an Article from the September, 1949 *Food Drug Cosmetic Law Quarterly*. Dr. Lehman and His Coauthors Are All Associated with the Food and Drug Administration, Except for Dr. Patterson, Who Was Formerly with Its Division of Food

Introduction

by

Arnold J. Lehman, M. D.

In the September, 1949 issue of the FOOD DRUG COSMETIC LAW QUARTERLY there appeared an article entitled "Procedures for the Appraisal of the Toxicity of Chemicals in Foods." The article, prepared by members of the staff of the Division of Pharmacology of the Food and Drug Administration, outlined toxicological studies in greater detail than was presented in an earlier publication.[1] It has been stated that this outline has been largely adopted by laboratories conducting investigations in the field of chemical food additives.[2] Other publications from our laboratories outlined procedures for investigating the toxicity of substances for application to the skin, which

[1] G. Woodard and H. O. Calvery, "Acute and Chronic Toxicity," 12 *Industrial Medicine* 55 (1943).

[2] H. F. Smyth, Jr., "Toxicology," 5 *Annual Review of Medicine* 349 (1954).

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would include cosmetics,[3] and for the laboratory and clinical evaluation of new drugs.[4]

Since the appearance of these articles a number of publications by others have discussed several aspects of the subject of toxicology. The Food Protection Committee of the National Research Council has issued a statement on the principles and procedures involved in a testing and evaluating program for intentional food additives.[5] General considerations have been presented by several authors.[6] Special techniques have been described to serve as range-finding experiments for the preliminary screening of promising candidates for food, drug or cosmetic use.[7] The efficiency of criteria of stress and the influence of diet on toxicological studies have also been discussed.[8]

[3] J. H. Draize, G. Woodard and H. O. Calvery, "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," 82 *Journal of Pharmacology and Experimental Therapeutics* 377 (1944).

[4] W. Van Winkle, Jr., R. P. Herwick, H. O. Calvery and A. Smith, "Laboratory and Clinical Appraisal of New Drugs," 126 *Journal of the American Medical Association* 958 (1944).

[5] "Principles and Procedures for Evaluating the Safety of Intentional Chemical Additives in Foods," a statement prepared by the Food Protection Committee, National Research Council, November, 1954.

[6] Smyth, work cited at footnote 2; A. C. Frazer, "Pharmacological Aspects of Chemicals in Food," *Endeavor*, Vol. XII, January, 1953; J. M. Barnes and F. A. Denz, "Experimental Methods Used in Determining Chronic Toxicity," 6 *Pharmacological Reviews* 191 (1954); B. L. Oser, "Gaging the Toxicity of Chemicals in Foods," 29 *Chemical and Engineering News* 2808 (1951).

[7] W. B. Deichmann and T. J. Le Blanc, "Determination of the Approximate Lethal Dose with About Six Animals," 25 *Journal of Industrial Hygiene and Toxicology* 415 (1943); A. C. Bratton, Jr., "A Short-Term Chronic Toxicity Test Employing Mice," 85 *Journal of Pharmacology and Experimental Therapeutics* 111 (1945); C. C. Smith, "A Short-Term Chronic Toxicity Test," 100 *Journal of Pharmacology and Experimental Therapeutics* 408 (1950); H. F. Smyth, Jr., and C. P. Carpenter, "The Place of the Range Finding Test in the Industrial Toxicology Laboratory," 26 *Journal of Industrial Hygiene and Toxicology* 269 (1944), and "Further Experience with the Range Finding Test in the Industrial Toxicology Laboratory," 30 *Journal of Industrial Hygiene and Toxicology* 63 (1948).

[8] H. F. Smyth, Jr., C. S. Weil, E. M. Adams and R. L. Hollingsworth, "Efficiency of Criteria of Stress in Toxicological Tests," 6 *Archives of Industrial Hygiene and Occupational Medicine* 32 (1952); R. H. Wilson and F. DeEds, "Importance of Diet in Studies of Chronic Toxicity," 1 *Archives of Industrial Hygiene and Occupational Medicine* 73 (1950).

It is not the purpose of the present communication to evaluate the merits of these and other proposals and suggestions, but rather to outline in as concise a manner as practical the techniques presently employed by the Division of Pharmacology. Since reprints of our earlier publications are no longer available and in view of the continuing demand for copies, it was decided to combine all of our procedures into a single article incorporating all of the newer developments which have occurred since 1949. As heretofore, the staff member in charge of the unit in the division most closely associated with each of the 11 aspects of the subject matter to be discussed has prepared that phase of the outline. Throughout, we have attempted to include detail consistent with requests we have received over the past five years for more precise descriptions of certain of our procedures. The subjects, in the order in which they will be presented, are: chemistry, acute toxicity, pharmacodynamics, biochemistry, range-finding and subacute toxicity, chronic toxicity, carcinogenicity, dermal toxicity, pathology, statistics and interpretation.

I. Chemistry

by

Wilbur I. Patterson

The chemical information to accompany a toxicity study of a food additive, drug or cosmetic should describe the complete chemical composition, including impurities. In those cases where complete analysis has not been attained, the chemical data should establish that the product made today will have the identical chemical composition of that made a year from now. To achieve either of these goals, quantitative and sensitive qualitative methods of analysis are essential. Impurities or minor constituents in a commercial product often have an importance, particularly from a toxicological standpoint, far greater than their amounts would indicate.

In addition to the knowledge about the chemical composition of a material for toxicological study, certain of its physical properties are

also important. Solubility is perhaps the most significant of these. In general, any material for use as a drug or cosmetic or in food will be soluble either in aqueous media or in fats. Its toxicological effects may depend upon the media in which it is administered to the test animal. The use and limitations of physical properties, such as melting point, boiling point, density, refractive index and some others, as criteria of purity and identity, are well known. In special cases a specific property may have unusual value, as, for example, viscosity in comparing different silicone preparations.

Necessity for knowledge of the stability of a material to air, light, and especially to the conditions under which it is to be used either as an ingredient of commercial food for man or animal, or as a test substance for toxicity studies, cannot be overemphasized. It is obvious that if there is any detectable chemical change in the test substance between the time it is added to the test media and the time it is introduced into the test animal, the results may be misinterpreted in the sense that they may be due to a compound whose presence may not even be suspected. In chronic-toxicity studies it is common practice to make up enough diet at one time to last for one or two weeks, or even longer. With small concentrations of certain susceptible substances, the whole amount may have disappeared, or have been changed into something else in this time. No matter how complete the information on chemical composition of the original material may be, if it is unstable under the conditions of the experiment, even additional toxicological studies on one or more of the breakdown products may be indicated.

On theoretical grounds, toxicity of a chemical additive to food should be determined on whatever form the chemical is in at the time it is ingested in the food. It is a well-known fact that an antioxidant, when added to food to protect some valuable ingredient from destruction, is itself slowly oxidized to one or more other compounds during storage of the food in the presence of air. Before carrying out a toxicity study on a problem of this nature it would be necessary to identify the product formed by air or other agent.

Particularly appropriate examples of food additives which change after the addition are found in the pesticide field. Certain relatively simple organic phosphates cannot be detected on some plants 24 hours after application. Thus, the chemistry of additives to food is con-

cerned not only with the compound originally applied, but also with potential transformation products following its application or addition to the food.

In the desire to eliminate as many variables as possible in the time-consuming toxicity study of a material, the technical product is often further purified. Although such a step is highly desirable from the standpoint of fundamental knowledge, it may be of doubtful value, or even undesirable, if the purpose of the study is to determine the acceptability of the technical product for use as a drug or cosmetic or as an intentional or incidental food additive, because, after all, the safety of a commercial product must be judged from the toxicity of all its components.

For the relatively simple inorganic poisons, toxicity in most cases is the result of one element, and the effects vary little with the form in which the element is applied to food. This situation naturally leads to the hope that if one member of a class of organic compounds is found to be safe for a given use, other members will be equally safe; costly toxicity tests could be avoided. Such a hope is absolutely unreliable, as exemplified by the well-known case of ethylene glycol and propylene glycol. Seemingly insignificant changes in the structure of organic molecules may produce drastic changes in toxicity.

Thus, the relatively simple problem of earlier days, in which only the major components of the added material needed to be known, has now become a highly complicated chemical problem in which the impurities in the technical products, as well as the residues on the food, must be adequately identified qualitatively and quantitatively. The use of increasingly toxic chemicals has necessitated more sensitive quantitative chemical procedures at levels undreamed of a few years ago. Surprisingly enough, under the pressure of necessity, suitably sensitive tests for most additives to food are being developed. These need not be restricted to the chemical types of analysis; sometimes a biological test may be preferable where it is more specific or more sensitive than the available chemical tests. In residue studies on organic phosphate pesticides, a combination of paper chromatography with a spot test based on the enzyme cholinesterase has shown unusual value.

An example of a food "improver" is the so-called bread softeners, at least some of which are complex mixtures of compounds of similar

types, and to prove that any particular sample does not contain small amounts of potentially harmful substances is an extremely difficult analytical problem. In the case of nitrogen trichloride, a chemical long used as an additive to flour, the reaction of the chemical with flour gave a product in minute amount which made the flour toxic for dogs.

Numerous chemical compounds, both inorganic and organic, are added, or are proposed for addition, to feed for almost all domestic animals. The purpose is economic in that the use of the chemical increases the efficiency of food utilization, usually through an effect which classifies such use as that of a drug. However, the meat from slaughtered animals is a food and must contain negligible amounts of the drug which was originally fed to the animals, that is, storage of the drug in the edible portions of the animal should be zero.

Various new ingredients for food-packaging materials are being proposed. For the most part, the major component of these is a highly insoluble polymer. However, certain of those recommended for fatty foods are appreciably soluble in aqueous media and vice versa; thus, use of these for unrecommended applications could result in potentially dangerous food contamination. Proposed food-packaging materials should be tested for the extractability of all ingredients under conditions preferably more drastic than any which will be encountered in actual use. Details appear in a recent publication.[9]

According to our present laws, the tolerance for many chemicals in foods is zero. To the analytical chemist zero is an unrealistic figure and, in practice, "zero" becomes the limit of sensitivity of the analytical method. Such "zeros" have to be evaluated in terms of what is known of the toxicity of the chemical in question. Unless it is clear that the adoption of such a "zero" will result in no conceivable hazard to health, the sensitivity of the analytical procedure must be improved or the proposed use of the chemical abandoned. When the required sensitivity is less than 0.1 parts per million (p. p. m.), the solution of the analytical problem usually involves an extended investigation in which blanks and recoveries for the proposed method may become just as important as sensitivity. Sometimes experimental application of the chemical at a level several times that which will be recommended for practical use is helpful.

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[9] A. J. Lehman and W. I. Patterson, "F & D A Acceptance Criteria—Basic Considerations in Determining Safety of Chemicals Used in Food-Packaging Materials," 28 *Modern Packaging* 115 (1955).

A further point which is often overlooked but is nevertheless important, particularly for technical products, is preservation of a portion of the sample used in the toxicity study in case a comparison of its properties with those of later preparations may be needed. For those materials which are sensitive to oxygen and light, or are otherwise unstable under normal atmospheric conditions, this problem may not have a perfect solution; low-temperature storage in the dark and in an inert atmosphere such as nitrogen is best.

Summary.—In the study of the toxicology of commercial products for food, drug or cosmetic use, considerable chemical information should be supplied, along with the data on toxicology. This will include the complete chemical composition and evidence for the constancy of composition along with the usual physical properties, including solubility in aqueous and fatty media. Stability under the recommended conditions of use is particularly important. It is essential that adequate analytical methods be available for the major components and the impurities in the original commercial product and also for these substances in foods and biological tissues. For products such as pesticides, data to show the maximum residue from use in the recommended manner, and preferably also from experimental lots treated under exaggerated conditions, are necessary. In the case of chemicals which may change in composition after application to the food, a method must be available to measure the toxicity of the residues.

II. Acute Toxicity

by

Bernard Davidow and Ernest C. Hagan

The second consideration in the evaluation of the safety of a chemical is its acute toxicity. By acute toxicity we mean the effect that a compound produces when given in a single dose or in multiple doses over periods of 24 hours or less. The most convenient expression of the acute toxicity of a substance is the ED50. This is the amount of the substance (usually expressed in milligrams or grams per kilogram of body weight) which, on the average, will affect one half of a group of animals of a certain species under specified conditions. This standard of comparison was selected because the dosage required to effect a response in 50 per cent of the animals is more reproducible than any other dosage.

Dr. Davidow

Mr. Hagan

Experience has shown that the ED50 will vary from species to species and with environmental conditions so that it is necessary to specify the conditions of the experiment. Species, route of administration, sex, age, weight range, and state of nutrition of test animals, as well as physical state of chemical, solvent and concentration, are the conditions usually specified.

Although the effect most frequently reported is mortality (LD50), an acute toxicity determination is not necessarily limited to the estimation of the lethal dose. Any toxic manifestation such as emesis, convulsions, hypnosis, etc., may be reported in terms of ED50. However, for this section the discussion will be limited to mortality, even though the points raised are applicable to other toxic effects.

Because different species of animals and even various strains of the same species may differ widely in sensitivity to drugs, the LD50 should be determined on at least three species of animals, one of which should be nonrodent. An evaluation of these results will give a measure of the species variability, and thus permit the pharmacologist to estimate the toxicity of the test substance for man.

If all of the LD50's are of the same order of magnitude, it is usually safe to assume a similar LD50 for man. Cyanide is a good example of a chemical which has approximately the same LD50 for most species of mammals, including man. If, however, there is a wide variation of LD50's among species of test animals, the estimation of the probable LD50 for man is much more difficult. In some cases, the estimate can be based upon man's position in the phylogenetic scale in relation to the test animals. In other cases the estimate must be

based upon biochemical and metabolic similarities of man with the test animals. For example, a chemical that may produce methemoglobinemia as its characteristic effect can be tested in the cat or dog, known to yield this response similarly to man. Tests in monkeys, rats and rabbits, which are known to produce practically no methemoglobinemia in response to such chemicals as acetanilide and nitrobenzene, would obviously be of little value in estimating this particular hazard for man. Where adequate knowledge of similarities between man and the test animal is not available, it is safest to assume that man is at least as sensitive as the most sensitive species of animal tested.

Of all the factors which influence the LD50 of a drug, the route of administration is the one most apt to modify the toxic effects. For most chemicals, the oral and intravenous LD50's are sufficient. However, for drugs which may enter the body by some other route—for example, inhalation—the acute-toxicity determination must be designed so that the results are applicable to actual use conditions. Other factors which influence absorption and, thus, indirectly the toxicity are (1) the physical state of the drug—for example, dry chemicals are more slowly absorbed than those administered in solution; (2) solvents—for example, substances dissolved in oil are absorbed more slowly than those in water; (3) concentration of the drug—for example, concentrated solutions are usually more rapidly absorbed than the administration of the same amount in a more dilute form; and (4) presence of other substances—for example, suspending agents may hinder absorption partly by adsorption of the drug, partly by blocking its access to the absorbing surface. Food in the alimentary canal has this action. On the other hand, the presence of surfactants (surface active agents) may hasten absorption. In practice the chemical is usually given orally to animals which have been fasted overnight (water allowed ad libitum). The substance may be administered by stomach tube, capsules, or mixing with the animals' food. Where the recommended administration of the drug is by the parenteral route, the experimental administration of the drug (subcutaneous, intraperitoneal, intramuscular or intravenous injections) must be related to the recommended usage. In no case is it necessary or advisable to anesthetize the animals in order to administer the test dose. The relationship between the intravenous and oral LD50 usually indicates the extent and rate of intestinal absorption.

Although water is generally the most satisfactory solvent, there are many cases where the material cannot be adequately dissolved or suspended in this vehicle. Often, solution in vegetable oil or emulsification is satisfactory. The emulsifying agent currently employed in our laboratory is an alcohol-soluble lecithin (oil 15 per cent, lecithin 0.75 per cent, water to make 100 per cent, emulsified with a hand homogenizer just before administration of the test dose). Suspending agents are occasionally employed, but extreme care must be taken to avoid complications arising from the physical or pharmacological nature of the suspending agent. The suspending agent most frequently employed for oral use is carboxymethylcellulose. Solvents such as alcohol, glycerine, glycols, and the like should be avoided because of their own pharmacological actions. Because the LD50 of a chemical may vary somewhat, depending upon the concentration of the drug in the test dose, we have made it a practice to employ the most concentrated solution consistent with an easily and accurately measured dose. Exceptions to this rule may arise occasionally due to the irritating or corrosive effects, etc., of some concentrated solutions.

The irritating or corrosive property of drugs—their likelihood of forming abscesses—is of special significance when used parenterally. Their propensity for causing muscle damage is best evaluated by comparison with suitable controls (drug vehicle, other accepted injectables). By using the anterior and posterior portion of each side of the sacrospinal muscle of a rabbit, it is possible to make two injections of the test substance plus two injections of the control per animal. If properly injected, the entire dose, generally one milliliter, is retained within the body of the muscle and does not escape between muscles as when the thigh, for example, is used. The animals are sacrificed approximately five days after the injection, and the muscles are removed and fixed in 10 per cent formalin. In evaluating the lesion (after fixing), thin (three millimeter) slices are cut through the affected area so that the extent of the damage can be estimated (for example, 2.5 x 0.5 x 0.5 centimeters) and sections taken for microscopic examination.

It has been our experience that for oral administration, volumes of one to 64 ml./kg. of body weight for aqueous solutions or suspensions can be accurately administered and are well tolerated by the test animal. Oil solutions, however, are limited, because of their laxa-

tive effect, to 8 ml./kg. of body weight. These limitations may be circumvented, when necessary, by the administration of the test material in divided doses over a period of several hours.

Young adult animals, which have not been used for previous test purposes, are assigned to various dose levels with some element of randomization. Both sexes are equally distributed as to treatment in order to determine whether there is any unusual difference in response. It is also advisable to ascertain whether there is any difference in response in either younger (for example, rats at one month) or older (for example, rats at one year) animals.

There are numerous other factors of less importance which may cause unusual results in a few cases. Some of these are variation in environmental temperature, variation in crowding animals, variation with season and variation in diet, particularly in the larger animals. It is always advisable to choose a set of standardized conditions approximating normal.

The number of rodents used should be large enough to permit the quantitative estimation of the LD50 with a 95 per cent confidence interval of $\pm$ 20 per cent or less. This can be accomplished in most cases by the use of five or six groups of ten animals each, with dosages being spaced in a geometric progression so that one dosage group yields a 10-20 per cent mortality, another group an 80-90 per cent mortality, and at least one other group yielding an intermediate response.

In the interest of economy, the number of nonrodent animals per dose level may be reduced somewhat. On the basis of the information obtained in the rodent study, test doses in a geometric progression may be given to single animals. The direction of the test doses (higher or lower) will depend on the results achieved with the previous doses. In this manner, a reasonable approximation of the LD50 can be achieved with as few as 12 animals.

In order to facilitate the analysis of the relationship between dosage and mortality, the sigmoid curve produced by plotting the data on arithmetic scales is changed to a straight line by transformation of both the dose and response scales. This is accomplished by converting the dosage to a logarithmic scale and the percentage response to units called probits.[10]

[10] C. I. Bliss, "The Determination of the Dosage-Mortality Curve from Small Numbers," 11 *Quarterly Journal of Pharmacy and Pharmacology* 192 (1938).

Probits are probability units arranged on a scale of 10 so that the value 5 represents the level of 50 per cent response. The transformed data are plotted on ordinary graph paper. A provisional straight line which best fits the plotted points is drawn. The provisional line may, and in many cases should be, corrected by statistical computations. The LD50 is the antilog of the log dose of the point where this line intersects the probit 5 line. The computations involved in the more rigorous probit method of Bliss are time-consuming. Several papers have been published on simplifications and estimations of the probit LD50. The short-cut methods generally sacrifice part of the information contained in the data in order to avoid the lengthy computations. For the ordinary needs of acute-toxicity determinations, the Litchfield-Wilcoxon [11] simplified method of evaluating dose-effect experiments is suitable, if the data conform fairly well to the normal frequency distribution. This method employs a logarithmic probability graph paper [12] and a number of nomographs which simplify the calculations.

In addition to the estimation of the LD50, the statistical method employed should also permit the investigator to characterize the substance with respect to the slope of the dosage-response curve and provide the errors of these estimates.

The slope of the dosage-mortality curve is a measure of the change in mortality which occurs with a change in dosage. Very "flat" curves indicate that occasional instances of extreme susceptibility to the chemical may be expected, perhaps even at the levels of proposed use. On the other hand, a "steep" curve indicates little variability, and consequently permits a better estimate of a safe dosage. For example, diethylene glycol is a substance which in most species of animals gives rise to a "flat" dosage-response curve. This flatness was reflected in the human-poisoning cases with diethylene glycol in which some individuals died from very small doses, whereas others survived relatively large doses. In contrast to diethylene glycol is the experience with a toxin, occurring occasionally in clams and mussels, called gonyaulax toxin. Here the dosage-response curve is "steep," so that a quarter of the LD50 can be eaten with almost no risk of fatality.

In addition to recording the amount of drug required to kill a certain percentage of the animals, it is essential to note the type, time of

[11] J. T. Litchfield, Jr., and F. W. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments," 96 *Journal of Pharmacology and Experimental Therapeutics* 99 (1949).

[12] Codex Book Company, Inc., Norwood, Massachusetts No. 3128 or No. 32,376.

onset, severity and duration of all toxic signs or symptoms. The pupillary reaction, pulse regularity and rate, respiration type and rate, and general activity of the animal should also be watched. Gasping, salivation and anorexia, vomiting, unusual defecation, condition of the animal's coat, and abnormal posture should be recorded if they occur. Observation of the animals is usually continued for at least two weeks. However, if the onset of symptoms is delayed for several days, or the animals continue to appear sickly, a four weeks' observation period becomes necessary. It has been our experience that compounds which have a flat dosage-response curve and/or a marked delay in the onset of symptoms are frequently quite toxic upon long-term ingestion. Data of this sort frequently furnish the investigator with enough clues to enable him to design experiments to establish definitely both mechanism and site of action, and to select dosage levels for subacute and chronic feeding experiments.

Before a compound is subjected to a toxicity study, the chemical and physical properties and all available background information are reviewed. Generally, on the basis of the information available, the proper solvent and concentration may be selected for a preliminary toxicity determination. In cases where there is little or no previous information which would enable one to estimate the order of toxicity, a few exploratory doses at 0.6 log intervals are given to one male and one female animal on each dose. The exploratory doses follow this progression: 20 mg./kg., 79 mg./kg., 316 mg./kg., 1,260 mg./kg., 5,010 mg./kg.

If the onset of symptoms and death occurs within several hours, it is generally safe to proceed immediately with the second trial assay the next day. In cases where all ten animals live or all ten die, an additional preliminary assay using higher or lower doses is indicated. On the basis of the information developed in the preliminary assay, two additional doses, spaced 0.2 of a log interval apart, are selected. Two males and two females are treated on each dosage level. The dosages selected for the final assay are based on the results obtained in the second trial. An example is presented in Table 1. If the mortality ratios in the second trial indicate an extremely steep slope (0/4 and 4/4), the log-dose interval for the final assay should be 0.05 log units (501, 562, 631, 708, 794, mg./kg.), instead of 0.1.

Table 1

Typical Assay

First Trial Dose mg./kg.	Mortality	*Second Trial* Dose mg./kg.	Mortality	*Third Trial* Dose mg./kg.	Mortality
20	0/2				
79	0/2			398	0/10
316	0/2	501	1/4	501	2/10
1260	2/2	794	3/4	631	6/10
5010	2/2			794	8/10

When, through experience or background information, the toxicity of the compound can be estimated, saving of time and animals can be achieved. This may be accomplished by using four or five doses spaced at 0.3 or 0.2 log-dose intervals (depending upon the confidence the analyst places in his prediction) as a preliminary assay. Based on the data obtained in this preliminary assay, a final assay using ten animals per dose, spaced at 0.1 log intervals, can be set up.

As a rule, savings in time can be assured by the expenditure of more animals, and savings in the number of animals used can be achieved at the expense of the length of time necessary to complete the experiment (waiting upon the results of one dosage level before starting the next test dose). The scheme previously outlined is a reasonable compromise between time and number of animals expended. In any case, surviving animals are observed for a minimum of two weeks in order to catch instances of delayed toxicity.

A graphic and tabular summary of the data presented in Table 1, including symptoms, time of onset of symptoms, time of death, etc., is illustrated in Figure 1 and Table 2. The results obtained by the

Table 2

Summary of Typical Assay, Including Results Obtained by Different Statistical Treatments

Drug	XYZ	Animals	Rats, fasted, M & F
Dose	398 - 794 mg./kg.	Weights	100 - 150 g.
Concentration	5% in water	Route of Administration	Oral
Observations	Tremors and convulsions, onset 2 hrs., deaths 3 — 18 hours, equally toxic to males and females.		

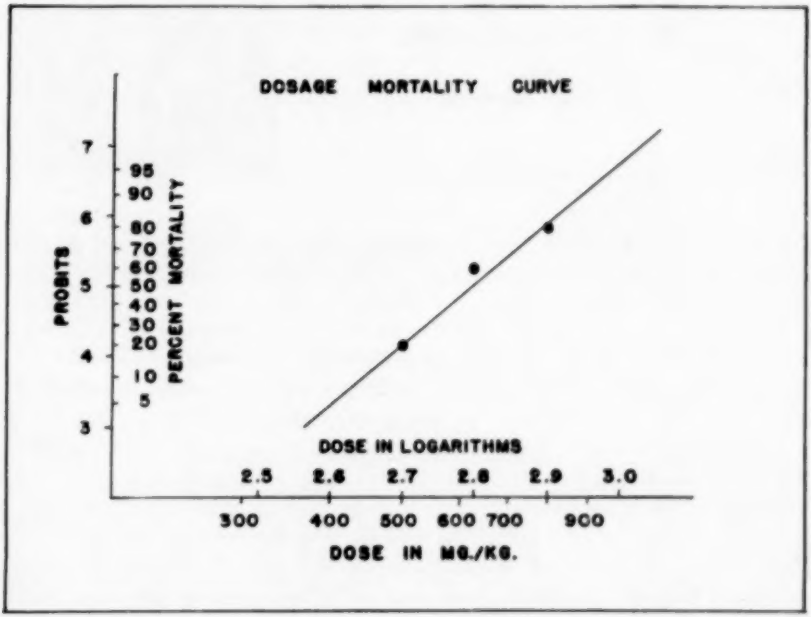
	Litchfield-Wilcoxon	*Bliss*
LD50	625 mg./kg.	622 mg./kg.
Confidence interval of LD50	558 -702 mg./kg.	548 - 706 mg./kg.
Slope function - Slope	1.25	8.7
Confidence interval of Slope function - Slope	1.1 - 1.4	4 - 13

different statistical treatments are in good agreement. (The apparent disagreement in values obtained for the slope function and the slope is due to the fact that they are expressed in different units.)

The interpretation of the acute toxicity data for the purpose of estimating a safe or therapeutic dose is not based upon a simple mathematical formula. Among the factors which are weighed in evaluating the data are:

(1) Adequacy of the data from a statistical viewpoint—sufficient number of animals employed so that the chemical can be characterized with respect to its LD50 and slope of the dosage-response curve and the errors of these estimates.

Figure 1



Graphic Presentation of Data Obtained from Table 1. Dosage Mortality Curve Based upon Probit-Log Dose and Showing the Equivalents in Percentage Mortality-Arithmetic Dose.

(2) Interspecies variation—susceptibility of man as compared to the experimental animals; generally, man is six times as sensitive

as the dog and ten times as sensitive as the rat to the toxic effects of drugs.

(3) Intraspecies variation—differences in susceptibility which normally occur in a general population (age, sex, state of health and nutrition, stress, etc.).

(4) Usefulness of chemical or drug—whether the benefits derived warrant the risks.

III. Pharmacodynamics

by

Geoffrey Woodard

Just as a chemical may be characterized or fingerprinted by its behavior with group reagents and by its physical properties as determined by spectrophotometric and solubility measurements, so too can it be characterized by a detailed description of its sites and mechanisms of pharmacological action in the animal body or, in short, its pharmacodynamic behavior. All too often in the pressure of events involved in obtaining data to support the safety of a chemical for use by man, the pharmacodynamic study is relegated to second place or forgotten entirely. In other cases, the pharmacodynamic study came as an afterthought when it became apparent that certain observations made during the course of the classical toxicity studies needed explanation. Logically, the study of the sites of action and the mechanisms of pharmacological activity should occur early in the investigation scheme for a chemical that will ultimately be used by man, whether it be in a food, a drug or a cosmetic.

Many advantages will accrue from a thorough pharmacodynamic study. An understanding of the duration of action or duration and height of blood levels following single doses provides a rational basis for dosage schedules in a drug use for the chemical, and provides some

help in planning the dosage schedules or methods of administration of the chemical in the toxicity experiments. The knowledge of the mechanism of action of a chemical leads directly to the development of specific antidotes for the treatment of possible poisoning cases. Furthermore, this knowledge also tells the investigator what to look for in the nature of side effects when the chemical is used for a drug; it tells the toxicologist what to expect in the chronic-toxicity experiments and what type of tests to use to best bring out the toxicity or lack of toxicity of the chemical; it tells the pathologist where to look with special care for tissue damage; and it tells the biochemist what biochemical systems would be most promising to investigate for the metabolism or enzyme poisoning action of the chemical. The development of biological assays when they are needed also logically follows an understanding of the pharmacology of the chemical. Finally, those cases in which a drug is contraindicated, and those cases where various drug combinations would be harmful or irrational, can only be predicted from an adequate study of the detailed pharmacodynamic action of the chemical in question.

There are many approaches to the specific study of the pharmacodynamic action of chemicals. In the majority of cases, these approaches employ the classical methods of pharmacology, which are well described in the literature and will not be discussed in detail here. Instead, an outline of the type of information desired is listed below:

(1) The effects of the chemical itself on:

(A) The central nervous system: excitation, depression, hypnosis, disturbance of temperature regulation, ataxia, disorganization of motor activity;

(B) The autonomic nervous system: stimulation or depression of either the sympathetic or the parasympathetic branches of the system in unanesthetized animals; the location of the sites of such activity in appropriate animal preparations, whether on the central control of the antonomic system, the ganglia, or the peripheral nerve endings;

(C) The cardiovascular system: specific action on the heart and the peripheral blood vessels, indirect action and the mechanism of such indirect action;

(D) The respiratory system: whether peripheral or central control of respiration, or an effect due to an interference with the acid-base balance of the body;

(E) The gastrointestinal and excretory systems: nausea, emesis, diarrhea, constipation, diuresis, antidiuresis. The reasons for any such activity, whether by direct irritant or depressant action, by autonomic nervous system activity or by direct pharmacodynamic action on the gut or kidney;

(F) The reproductive system: prolonged estrus or diestrus, spermatogenesis, contraction of uterus in estrus and diestrus;

(G) The special senses: taste, smell, auditory or optic nerve dysfunction; and

(H) The composition of the blood: blood sugar, nonprotein nitrogen, unusual blood pigments, clotting time, as well as the formed elements of the blood.

(2) The effects of the chemical upon the activity of other commonly used drugs. For this reason it is advisable to conduct a few experiments on:

(A) The duration of action or potency of action of selected common central nervous system depressants and excitants;

(B) The effect on the duration of blood levels or the rate of urinary excretion of common acidic and basic drugs; and

(C) The effect upon the absorption of essential minerals and vitamins from the gastrointestinal tract.

(3) The effects of the chemical upon selected enzyme systems. At the present time, there are only a few enzyme systems for which it has been clearly established that a chemical effect has much meaning for the pharmacological or toxicological behavior of a chemical. The importance of the cholinesterase and the carbonic anhydrase systems in the evaluation of the action of chemicals is well recognized. Effects of a few chemicals upon the carbohydrate cycle would appear adequate to explain their mechanisms of action. Although the importance of a variety of other enzyme systems to the proper functioning of the body is known, it is not clear at this time how much weight can be given to the effect of a chemical on one of these systems in the evaluation of the safety of such a chemical. Undoubtedly, the near future will see more and more emphasis given to the action of chemicals on a large variety of selected enzyme systems.

It should be quite obvious that both the design of the toxicological experiments and the evaluation of the data derived therefrom must be materially tempered by a consideration of the end use of the chemical

in question. Even when it has been decided that the end use of the chemical will be limited to that of a drug, additional considerations must be given to the design and evaluation of experiments, depending upon the specific instances in which the drug will be used. Following are some of the modifying conditions that relate to drug use.

In general, a drug is usually considered by a pharmacologist as being a poisonous substance. It is the specific poisonous action of the chemical that is made use of in the treatment of a disease or condition in man. The toxicological and pharmacological tests on a drug then are made for the purpose of guiding the physician or the patient in the safe and rational use of a poisonous substance in the treatment of a diseased condition which, if left untreated, would be more dangerous or inconvenient than the possible poisonous effects of the drug itself.

There are several circumstances that permit the relatively safe use of a poisonous substance as a drug. First of all, taking of the drug is usually by the free choice of the patient. It can be discontinued at any time if necessary because of toxic reactions. The drug is often given under the supervision of a physician who, by training and experience, knows what toxic signs to look for and how they may be treated or ameliorated. Drugs are carefully manufactured and labeled, and their strength and purity are rigidly controlled. The more toxic of the drugs are limited in their availability by sale only by registered pharmacists on prescription of a physician.

For a specific drug use, other considerations are necessary in evaluating drug safety. A drug used to treat a very serious diseased condition can be given a little more leeway in toxicity than a drug for a less serious condition. Some drugs are used only in institutional practice where laboratory facilities are available to follow the reaction of the patient to the drug. Some drugs are used for infrequent acute illnesses, while others are used over prolonged periods to treat chronic disease. A drug available for over-the-counter purchase by the user must have a better safety record than one limited to prescription dispensing. Where a known drug is already useful in the treatment of a diseased condition, it would be pointless to develop a new drug for this same condition which would be more toxic than the known drug unless it offered significant advantages. Finally, the proposed route of administration of the drug for a specific case necessarily alters the type of experimentation needed in the study of the toxicity of the drug.

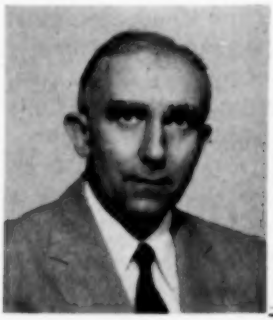
Because of the considerations mentioned in the foregoing, the experimentation involved should be altered to fit the circumstances. If the drug is used only in occasional acute illnesses, chronic experiments need be only one to three months in duration. If it is to be used for chronic disease, the chronic experiments should be increased to six months' or a year's duration. The method of administration of the drug should follow as close as practicable to the proposed method of human use. Dosage levels for drug chemicals in chronic experiments should be chosen so that they include a low dose of the same order of magnitude as the maximum human dose, and a high dose sufficient to cause definite toxicity and/or pathology. It is still advisable to use one rodent species and one nonrodent species in the chronic experiments. Blood and pharmacodynamic studies become especially important in the investigation of a drug chemical. Since the usual toxicity studies for a candidate drug are designed to determine a tolerated level of toxicity rather than to determine the amount that shows no toxicity whatsoever, the numbers of animals needed in each experiment can be materially reduced from those used in the study of a food chemical. For example, it is usually sufficient to use about ten or 12 rats per level for three dosage levels plus control animals for the chronic rat experiments. The numbers of dogs, however, cannot be materially reduced, since the number used in food-chemical experiments is already so low that to use fewer dogs would lead only to inconclusive results. There is, however, an additional experiment that should be run on candidate drug chemicals wherever possible. A drug already used for the specific diseased condition under study should be run in parallel in the toxicity experiments as a sort of positive control.

The use of a chemical for a drug sometimes involves certain additional experiments not usually done if the chemical is to be limited to food use. These additional studies, where applicable, should include:

(1) Reactions or irritation at the site of an injection drug;

(2) Blood or tissue level studies to determine the length of time that a significant amount of the drug remains in the body in order to establish rational and adequate dosage schedules; and

(3) Biological control procedures designed to exclude from the finished drug such harmful impurities as pyrogens, bacteria, decomposition products and accidental additions.



IV. Biochemistry

by

Edwin P. Laug

In the over-all evaluation of a compound which may be added to food, the biochemist, so to speak, occupies a "halfway" position in the course of the procedures. First, the chemist must characterize the compound with information on physical and chemical constants, as well as devise sensitive methods for its detection. Second, the toxicologist, on the basis of acute and subacute experiments, must decide *how* toxic the compound is. The biochemist then must determine *why* the compound is toxic. Finally, with the above information at hand, the toxicologist is able to plan the long-term experiments more intelligently.

The biochemist in determining *why* a compound is toxic is chiefly concerned with studying the absorption, excretion, storage and metabolism of the compound. It is proposed to discuss these properties particularly as they may relate to the other aspects of the problem stated above.

Absorption and Excretion.—These are so interdependent that it is difficult to discuss them separately. The same may also be said about metabolism and storage. For instance, the proportion of an administered test material that is excreted by an animal can be shown to be dependent on (1) how much is absorbed, (2) how much is destroyed or modified by metabolism and (3) how much is stored in the tissues. The resultant dynamic equilibrium can be further modified by the level at which the test compound is administered. For example, absorption of a compound from the gastrointestinal tract will be affected by (1) solubility, (2) permeability of gut wall, (3) irritation to gut wall and (4) destruction or modification by the fluids of the gastrointestinal tract.

While it is true that ultimately all information about absorption must bear reference to the oral route of entry, it is frequently necessary to rule out the above modifying influences by administering the compound intravenously. Actually, in any exhaustive biochemical study of a new compound, it is recommended that parallel oral and intravenous administrations be carried out. It is then possible to express these as ratios, thus: blood level (oral)/blood level (intravenous) or urine level (oral)/urine level (intravenous), etc. Such a ratio could be designated as an absorption ratio, and would furnish interesting information about the behavior of the compound. It could, for example, approach unity if the compound was able to enter the organism from the digestive tract with great facility.

Quantitation of fecal excretion following oral administration furnishes little significant information except to demonstrate roughly the degree to which a compound has *not* been absorbed. True excretion via an outward movement through the gut wall or the bile can of course be determined only when the test compound has been given intravenously. The intestinal avenue for elimination of substances from the bloodstream is probably no less important than the urinary, but the technical difficulties and the special conditions under which it must be studied have restricted its application.

Finally, no evaluation of absorption and excretion is complete until the stress limit has been determined. Stress limit can be defined as the oral dose which produces a deviation from the normal pattern of absorption, excretion and metabolism. Usually, but not necessarily, it is accompanied by an acute toxic response. The so-called "normal" pattern is generally established by administering several doses within the range in which normal usage of the compound is anticipated. The spread of values encompassed by these limits offers a convenient evaluation of a "factor of safety" for the compound under study.

Storage.—After determining the major avenues of excretion, the rate at which excretion occurs, and whether or not the original compound or a metabolite thereof is involved, the biochemist next poses this question: Is the compound stored somewhere in the organism? If so, then a further series of questions must be answered: (1) Is the compound stored as such? (2) Is a metabolite of the compound stored? (3) What, if any, is the significance of their relative proportions; should both be present? (4) How stable is the storage depot? For a preliminary survey of possible storage depots, the following tissues and

organs should be assayed: skin, fat, muscle, bone, kidney, liver, spleen, brain, adrenals, gonads and lungs. From extensive experience along these lines it can be said that the most likely depots of storage are kidneys, liver, bone and fat. Storage may be sufficiently slow that the results of a single administration are not readily determinable; hence it is advisable to extend daily administration over a period of at least a week before examining tissues. Paradoxically, storage may be regarded as a potential hazard or as a potential "escape" for the organism. Tissues most likely to be considered in the latter category are bone and fat. Lead storage in bone and DDT storage in fat may be cited as examples. Here it is conceivable that temporary removal from the bloodstream via these avenues may relieve, at least temporarily, fatal peaks of concentration. However, it has also been amply shown that the release of these stores into the bloodstream may constitute a hazard as, for example, rapid metabolism of DDT-laden fat stores in response to starvation, and calcium and pH disturbances in leaded bone. With regard to storage in the kidney or liver, experience has shown that this is always a hazard because it results in demonstrable cell pathology and the eventual destruction of the excretory function of these tissues.

Metabolism.—Metabolism or partial conversion of a compound may occur before, or coincidental with, its absorption from the gastrointestinal tract. This is one of the reasons why, for complete evaluation, the dose should be administered both orally and intravenously, the effect on the organism should be compared and the identity of the metabolite should be ascertained. While a clear distinction between external and internal metabolism should be made, it is believed that internal metabolism—that is, conversion after absorption—is the process which holds the greatest interest. The organs most frequently involved are the liver and the kidneys. To establish the site of metabolism for these or any other tissues, in-vitro incubation studies are very useful. Since this procedure leads naturally to only a preliminary or gross evaluation, cell structure should be maintained as intact as possible by the use of thin tissue slices. Two avenues of procedure are open:

The first is intravenous administration of the compound, followed by prompt removal of the tissue in question, and its incubation, either intact or in slices, with periodic assay for either disappearance of the original compound or the appearance of a derivative. This procedure

insures that the compound enters the tissue and is distributed therein to its ultimate cellular units via the bloodstream, but it is difficult to quantitate because it can never be determined accurately how much substance was present initially.

The second procedure is addition of the compound to tissue homogenate or slices. Here the amount added can be easily determined, but the question of how to add the compound—that is, in what kind of solvent, etc.—becomes difficult. It need hardly be emphasized that the more nonspecific disappearance of a compound from a homogenate may involve a dangerous interpretation. It is better to obtain evidence of disappearance via the reappearance of a changed compound or metabolic fragments.

In addition to the tissue incubation studies, another approach should be mentioned that has been successfully used in our laboratories. This consists of "poisoning" the organ which is suspected of being involved in the excretion or metabolism of the compound. For example, it has been shown that small doses of carbon tetrachloride are effective in embarrassing the liver so that it is no longer able to metabolize methoxychlor. When this occurs, methoxychlor, which is ordinarily quite nontoxic, becomes as toxic as DDT and, contrary to its usual behavior, is heavily stored in fat. Also, a series of metabolites which ordinarily escape into the urine are diminished in number.

All of the studies mentioned so far concerning metabolism have had to do with the gross tissue. These studies are, in fact, merely "curtain raisers" to the ultimate job that the biochemist must now undertake. This is to determine the enzymes or enzyme systems that are involved. These systems may enter the picture in two ways:

(1) Enzymes that effect the modification or destruction of the compound.—Most frequently this action produces metabolites of the compound which are less toxic than the original. However, it has also been shown that metabolites may be produced which are more toxic than the original substance. Experience in our laboratories has shown this to be the case with octamethylpyrophosphoramide and heptachlor. These findings pose special problems for the biochemist and toxicologist, and emphasize even more the potential hazards from ingestion of compounds which are completely foreign to the body.

(2) Enzymes whose action is destroyed or inhibited by the compound, and as a result of which serious dysfunction of the organism occurs.—This is the *res ultima,* the "why" of toxicity. The uncovering

of such information can lead very promptly to the perfection of useful antidotes. Much progress has been made in connection with tetraethyl pyrophosphate, parathion, and a host of others of like nature, because of the early discovery of their anticholinesterase action. It is a wry fact to note that in the case of DDT, even though the compound has been widely used for ten years, no such dysfunctioning enzyme system has yet been found, which fully explains the observed toxicity.

Occasionally, information helping to establish the safety of a compound can be demonstrated best by determining the metabolic pathway. This is especially true of compounds of very low-order toxicity and structurally similar to, or conjugates of, normal food constituents. Demonstration that the pathway of metabolism is identical with that of the normal constituent and of the absence of deleterious effects before this metabolism occurs provides valuable information for interpretation of safety.

The techniques applicable to this phase for investigation are specific for each compound. They usually require the use of compounds tagged with radio-isotopes in a definite position, and studies on the isolation and identification of metabolic products.

In general, the results should indicate the rate of biological conversion of the compound into a normal metabolite and that this additional supply does not place a burden upon the system. The latter can be demonstrated by following the eventual disposition of the tagged metabolite in relation to the normal pathway. Such a study does not necessarily eliminate the need for subacute or chronic toxicological studies.

V. Range-Finding and Subacute Toxicity

by

John P. Frawley

"Range-finding experiments," as the term indicates, are designed to provide a reasonable estimate of the maximum dosage an animal

will tolerate on repeated administration. It is a rapid empirical elimination of inappropriate dosages prior to more extensive subacute and chronic experiments.

Subacute experiments are designed to pinpoint the maximum tolerated, and the minimum grossly toxic, doses; establish the biological nature of the toxic effects; determine variation in species sensitivity; and permit decisions as to the desirability and exact design of chronic studies. Subacute experiments should extend over a period of time at least equal to 10 per cent of the average life span of the species.

Range-Finding Experiment.—This phase of a toxicological investigation is a necessary preliminary to the initiation of the subacute experiment. Its results provide the order of magnitude of dosages to be selected for the latter. The principal considerations for the phase are (1) selection of species, (2) selection of route of administration, (3) selection of dosage and (4) observation.

Selection of species.—Unfortunately, to date, there is not available a good screening technique for determining the species most closely approximating the human in response to a given substance. Thus, at the very beginning of the preliminary studies, the investigator is placed in a position of making a decision vital to the successful evaluation of the safety of a given compound.

Two general principles are available to guide the investigator in the selection of his species:

(1) One species should permit accurate comparison of the toxicity of the test compound with known compounds tested in the same species as well as in man. This permits indirect estimation of the toxicity of the test compound to man through other compounds. At the present time, use of the rat permits this comparison better than does that of any other experimental animal.

(2) Another species, other than rodent, should be selected on the basis of its particular similarity to the human in relation to the suspected physiological and metabolic mechanisms affected by the test compound. If no valid basis for such a prediction is available, safety is best assured by selection of the most acutely sensitive species as the second test animal.

Selection of route of administration.—The basic rule for selecting the route of administration is that the route should be as nearly identical to the proposed human usage as possible. In the case of food

additives, the compound should obviously be incorporated in the diet of the animal. In the case of oral drugs, daily administration by capsule to larger animals is indicated. Drugs intended for parenteral use should be so administered during experimentation. If more than one route of administration is to be proposed, enough work should be done on each route to define the differences in toxicity.

It is not always possible to administer the test material to animals by the proposed route. Frequently, in feeding experiments, the animal will refuse the test material, and administration by stomach tube is necessary. However, in most cases, the animal can be conditioned to the off-flavor, smell, etc., by gradually building up the dietary concentration to the desired level.

In the discussion of acute toxicity, a preferred technique for short-term testing of local irritation on intramuscular injection, using the large back muscle of the rabbit, was mentioned. For range-finding and the later subacute experiments, administration of parenteral preparations will necessitate the use of the thigh and shoulder areas in rotation. A number of species may be used, but the dog is perhaps the most convenient. Again, as with the short-term technique, fixing of the injected area *in toto*, followed by thin slicing, gives the best demonstration of the extent of the tissue damage.

Selection of dosage.—Previous to the selection of dosage, a review of the acute toxicity should be made. This review should give consideration not only to lethal dose, but to the variation in animal response to the same dose and the slope of the dosage-mortality curve.

After determining an approximate LD10, a small group of animals should be administered this dose every day for two weeks. Three similar groups of animals should receive doses decreasing in size by 0.5 log units with each group. For example, if the estimated oral LD10 in rats of a food additive is 500 mg./kg., two male and two female rats should receive in their diets concentrations which will furnish a daily dose of 500, 160, 50 and 16 mg./kg. This would be approximately 6,250, 2,000, 625 and 200 p. p. m.

Experience with selection of dosages will permit the experimenter to choose dosages without adherence to a strict empirical protocol. But if nothing is known regarding repeated administration, this technique will usually be satisfactory.

In the case of larger animals, such as dogs, it is usually more economical to select the dosage after the toxic dose in other species

has been determined. Using this as a yardstick, one or two dogs can be risked on the lowest killing dose and the next lower dose.

Observations.—Since the range-finding experiment is designed to determine the approximate maximum tolerated dose preliminary to a subacute experiment, the most important observation is death. Since these experiments are of very short duration—two weeks—a death at any dosage level is sufficient justification to eliminate that dosage range for subsequent long-term studies. Additional observations should include evidence of the acceptability of diet, loss of weight, and symptoms.

In general, the range-finding study should furnish an approximation of the highest tolerated dose. This is represented by the logarithmic mean of the lowest killing dose and the highest nonkilling dose. For example if 625 p. p. m. kills no animals and 2,000 kills two out of four, 1,100 p. p. m. would be a reasonable approximation of the highest tolerated dose and the highest feeding level for subsequent studies.

Subacute Experiments.—Subacute experiments should not be considered as preliminary. Properly designed and conducted, the subacute experiment will provide the majority of the basic observations upon which the decisions for acceptability of use and commercial production will be based. Even when chronic experiments are later conducted, the observations made should be an outgrowth of initial suspicions or discoveries of the subacute phase. Therefore, a concentration of effort should be made in the subacute experiment to provide the best available screening for deleterious changes.

The subacute experiment should start in the library. A thorough understanding of the chemical nature, purity and stability of the compound, the biochemical reactions of specific groups, the physiological activity of similar compounds, and techniques for measuring suspected chemical and biological changes is essential to the proper design of the experiment. In fact all the considerations discussed in the chemistry section should be reviewed before starting the subacute phase.

In general, the subacute experiment should define the biological activity of a compound and provide a reasonable estimate of the lowest dosage required to produce the effect. The biological activity of a compound may be reflected in a change in the appearance of the animal or a part of the animal (*organic change*) or a change in the activity of the animal or a part of the animal (*functional change*). Frequently,

the subacute experiment has been limited to organic changes—those detectable from growth curves, organ weights, hematology, and gross and microscopic pathology. However, experience has taught the inadvisability of limiting studies to the detection of this type of change. Functional changes have proved to be the only index of subacute poisoning by many compounds. Thus, a complete subacute experiment must investigate both realms of change and estimate the dosage required to produce the change.

The proper design of a subacute experiment can result only from experience, insight, ingenuity and, in some cases, serendipity. Occasionally it is possible to predict the physiological activity from structurally related compounds, and the experimental design can be based on knowledge of these compounds. However, many compounds do not permit this sage judgment at this stage of investigation. Thus we must rely on as many screening techniques as possible to detect the type of toxicological change that occurs. These techniques are still in their infancy, particularly for detecting functional change, and as more basic research on these methods is conducted, the techniques and ideas contained herein will become outmoded.

(1) Organic phase.—This phase of a subacute experiment is the natural sequel to the range-finding study previously described. Its purpose is to determine what gross and microscopic changes take place, and at approximately what dosage level.

The mechanics of this phase of a subacute study can be more easily described than those of the other phase. The techniques are all "tried and tested," and apply to the great majority of compounds.

The range-finding tests have established for the investigator an estimate of the maximum tolerated dose by the particular route of administration. This dosage is now the basis of a new series of doses decreasing by approximately 0.3 logarithmic units from the maximum tolerated dose. Since relatively little is known of the cumulative toxicity at this point, the more dosage levels employed the better the chance of determining the lowest level of effect. Unless other evidence is available to guide the investigator, five dosage levels, plus a control, are considered a minimum for small animals, such as the rat. Selection of dosages for larger animals, such as the dog, can be aided by awaiting some of the findings of the small-animal studies, but should eventually include at least three dosage levels plus a control group.

The minimum number of animals used in a subacute experiment should be five of each sex for each dosage level for small animals, such as the rat. These animals should be weanlings of a known strain and colony. The animals need *not* be assigned according to litter throughout the experimental and control groups, since subtle inherited susceptibilities will not be demonstrated within the duration of a subacute experiment. However, the animals should be carefully assigned to groups in a manner which will assure an even distribution with respect to initial weight and age.

In the case of larger animals, such as dogs, at least two males and two females should be placed on each dosage level. These animals should also be from a known species and colony, and they should be started at a young enough age to permit evaluation of any possible effect of the compound on growth. Even distribution according to age and weight is also indicated.

The length of the subacute experiment, as previously indicated, should be at least 10 per cent of the animal's normal life span. In the case of mice and rats this is between two and three months, and for dogs between one and two years. As an arbitrary period, three months in the case of rats and one year in the case of dogs has become relatively standard. During this period, observations on each individual animal should include rate of growth, food intake, general appearance and behavior, complete hematological examination, and blood chemistry. Many other tests can be applied to these animals, but their selection usually is based on gross symptoms and pathology observed during the course of the experiment. For example, if an animal on the highest dosage dies during the second week with obvious liver or kidney damage, specific tests for this organ function should be applied to a sufficient number of surviving animals to evaluate the effect.

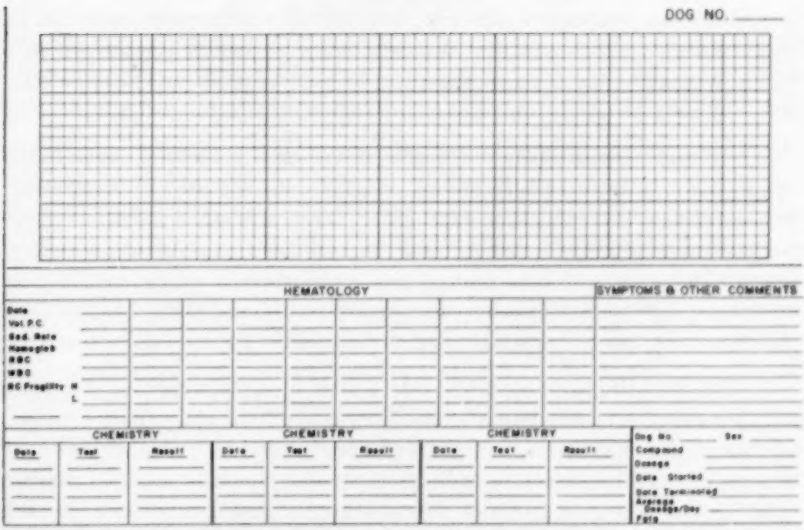
The blood chemical and hematological examinations on larger animals, such as dogs, are particularly useful, since pretreatment values permit use of each animal as its own control and, consequently, the detection of more subtle changes. We recommend a minimum of three pretreatment examinations, followed by test sampling at two weeks, one, three, six and 12 months for hematological studies. Most chemical observations should include at least five pretreatment values.

A typical chart used in this laboratory for recording data on subacute dog experiments is shown in Figure 2. The graph at the

top of this chart is used to plot growth rate and food intake at weekly intervals for three months prior to experiment and for the 12-month experimental period. We employ beagles as our dog strain, and the period of test is usually from six to 18 months of age. All dogs are vaccinated at three months of age against distemper and infectious hepatitis to minimize extraneous mortality. Each animal is individually housed and provided commercial dry dog food and water ad libitum. A weekly bone is offered each dog to help maintain oral hygiene.

At the termination of this organic phase of the subacute study, the animals are sacrificed, autopsies performed on all animals, major organs weighed and tissues preserved for histopathological investigation. Techniques for handling these tissues are described in the "Pathology" section of this paper. Other tests of enzyme activity or storage of the chemical compound should be performed on these tissues as indicated by the results of studies in the other phase of the subacute experiment.

Figure 2



Individual Record Sheet for Dogs on Subacute Experiments.

(2) Functional phase.—The previous phase of study is designed to detect gross and microscopic changes. Since all compounds do not exercise their biological activity in this manner, other studies must be designed to detect the changes that occur in the various biochemical systems of the body.

Many techniques have been developed for determining the effect of a compound on various biochemical systems of the body. However, it is not feasible, for example, to study the effect on every known enzyme system of the body. Screening tests must be available for studying these systems in classes and thus eliminating the majority of unaffected systems in a reasonable period of time. Unfortunately, research on such screening tests is in its infancy, and many effects will go unnoticed until they are perfected.

Fortunately, many compounds are selected for use and study because of their known effect on specific functions. This is particularly applicable to chemicals selected for potential drug use and certain insecticides. Here the functional effect is known and a study of the degree, permanence and significance of the effect can be readily undertaken.

Once the functional effect is established, the experiment should be designed to determine the lowest dosage at which this effect will occur, how dependent upon duration of administration is this effect and how long the effect persists after administration has ceased.

The selection of species for this phase of a subacute experiment can frequently be based on sound judgment in regard to the specific function to be studied. For example, a compound that causes central depression should be studied with regard to a species in which a change in emotional behavior is readily detected, as in the dog or monkey. Likewise, an anticholinesterase compound should be studied in a species closely approximating the human response, as in the dog.

The animals employed for this phase of a subacute experiment may frequently be the same animals employed for the organic phase. Chemical and enzymatic studies can be performed by venipuncture and biopsy of certain nonvital organs, provided control animals receive exactly the same treatment. More complicated procedures should be performed on separate animals to avoid confounding the interpretation of the organic phase.

In the case of enzyme inhibitors, techniques have been developed for studying the cumulative effect of compounds affecting the cholinesterase system. Although the experimental design may vary with other enzyme systems, the basic objectives are the same. The following design should, therefore, serve as a guide in studying the changes in any specific enzyme system.

The effect on the enzyme system of various organs should be studied on small animals, such as rats. This is accomplished by analyzing these systems at the higher tolerated dose. When the tissues for a study have been selected—as plasma, erythrocytes, brain or diaphragm—a series of dosages decreasing by approximately 0.3 logarithmic units are administered to groups of approximately 30 rats of the same sex. Five animals from each dosage level, including controls, are sacrificed for study at two, four, eight and 12 weeks. Administration of the test compound to the remaining animals is ceased and the animals are sacrificed at one and four weeks thereafter. If the system selected for study can be examined without sacrifice, much smaller groups of animals can be used. This design should permit the experimenter to determine the lowest level of administration which causes a significant change in the enzyme system. It should also reveal the degree of accumulation in the effect with repeated administration—and thus aid in the evaluation of desirability of conducting chronic studies. The reversibility of the effect is important in interpreting the hazard of intermittent administration.

In the case of cholinesterase inhibitors where plasma and erythrocytes provide the most sensitive index of cumulative effect, the dog is better suited for the final test animal, since its response more nearly approximates that of the human. Small numbers of dogs can be used in this case, since venipuncture will permit use of each animal as its own control. Five weekly predosing levels on each animal will provide a satisfactory control value and standard deviation for each animal. This technique can also be followed on rats and mice, using micro procedures, if blood is the tissue selected for study.

In general, the functional phase of the subacute experiment is designed to supplement the morphological studies. Together, the changes in the appearance and function of an organ and the changes in the appearance and chemistry of a cell can be evaluated. Since unchanged gross and microscopic appearance does not preclude the

presence of chemical changes, both approaches are frequently necessary for the proper evaluation of safety.

Summary.—Procedures are outlined for investigating the biological activity of a compound following repeated administration. Each compound must be considered individually for intelligent experimental design. The techniques employed should determine the effects of the compound—whether organic or functional. The lowest dosage level at which these effects occur should be determined. Evaluation of these effects in relation to dosage and use should permit a definite conclusion in regard to safety or indicate the desirability and exact design of longer-term experiments.

VI. Chronic Oral Toxicity

by

O. Garth Fitzhugh

Long-term tests are conducted on the premise that the possible effects of a lifelong ingestion of a chemical by man cannot be predicted with tests less stringent than lifelong feeding in a short-lived animal and on extended feeding in another species. Such tests are used to evaluate a new substance proposed for addition to food; a pesticide which, in its use, contaminates food; and a drug proposed for daily use over a period of several years. Necessarily, we are dealing with the hazard of human exposure to small amounts of a chemical for a long time. The effects of such exposures may differ markedly from an exposure to a large amount of a chemical for a short time. Some chemicals in large dosages show morphological changes in experimental animals within a few days; others show similar changes only after several months of feeding. Large dosages for a short time may affect one organ, whereas small dosages for a long time may affect another organ; or large dosages fed for short periods may produce one type of damage in a particular organ, whereas small dosages

fed for a lifetime may produce a different change in that particular organ. Cumulative changes, such as kidney damage, may occur within the first year, with some compounds, or may not occur until well into the second year of a rat-feeding study, with others. Compounds may show essentially the same morphological changes in subacute and chronic studies, but one may cause an increase in mortality towards the end of a lifetime. Finally, there is at present no substitute for time in evaluating the carcinogenic activity of a chemical, especially a low-grade effect.

The purity of the sample is of prime importance to the toxicologist. Ideally, the examination of the toxic properties of a chemical is done with a pure specimen of the material; however, since absolute purity is rare, one must select the best available sample (see the "Chemistry" section). When a commercial mixture of several ingredients is used, it is sometimes necessary to study the toxicity of the entire mixture along with that of each known entity. The choice of the material to be studied relates to the actual material to which the human will be exposed, and each experiment is designed to test the hazard of this exposure. Whenever the chemical under study varies in any degree in purity or physical characteristics, a sample large enough for the whole study should be selected before the experiment is started. It is the practice in our laboratory to make the entire study on a single manufactured batch of the chemical.

In the selection of a species of animal for a lifetime study, the albino rat has proved most adaptable. It is a well-standardized animal and has a life span of from 18 months to three years. Since some strains of the albino rat have a greater life span than others, it is advisable to sacrifice all remaining animals at some arbitrary time, such as two years. A nonrodent, such as the dog or monkey, should be used for the second species. An ideal experimental period for this second species would be throughout its growing period and through most of its adult lifetime. For the dog this means a period lasting from five to ten years. Obviously, such an extensive study is unreasonable for most chemicals that are proposed for food or drug use, and only in instances of suspected carcinogenicity is such an extensive study justified. It is very important, therefore, to know something about the nature of any expected harmful effect of the chemical before the chronic study is started. This may show, as in the case of some

organic phosphate insecticides, that long-term chronic-toxicity tests are not the best means to measure the chronic hazard of the chemical or it may show that other modifications of the procedure—such as reproduction, carcinogenic, etc., studies—are needed. Since the major question raised concerns the public-health hazard that may be associated with the eating of materials for the major part of a lifetime, in most instances the feeding of the chemical to experimental animals over a protracted period is the basic procedure to follow. For this procedure, numerous studies in this laboratory have shown that a two-year chronic study in rats and a one-year subacute study in dogs are suitable.

An appraisal of the data from the subacute toxicity investigation will permit the setting up of a well-planned chronic-toxicity experiment. If the lowest dosage level in the subacute study in rats and dogs has produced no demonstrable effect, the compound may be regarded as promising for a thorough investigation. On the other hand, if any dosage level in the subacute experiment has produced an effect, this effect must be weighed against the proposed use of the chemical. It is the purpose of the chronic-toxicity experiment not only to find a "no-effect" dosage level for the chemical but also to find a dosage level, if any, that produces a menacing effect on the animal as a whole or on one or more vital organs. The exact concentrations of the proposed chemical which should be added to the various diets in the long-term study will depend, therefore, upon the results of the subacute feeding. The lowest dosage level is adjusted so that no damage can be expected to occur within the experimental period.

In the rat experiment, at least four groups of animals are employed, each consisting of a minimum of 25 males and 25 females. In our previous outline of procedures for testing chemicals in foods we suggested fewer animals per group; however, this was based on our experience with a larger number of dosage levels. We had used, on the average, about seven dosage levels, or from 150 to 200 experimental animals. This number appeared sufficient; however, when the number of groups was reduced to four, the total number of animals proved too few for an adequate interpretation of results, unless the number per group was increased as indicated above. As explained in the section on statistical evaluation, any selected number presents a risk, and this risk must be evaluated in terms of the possible hazard from the use of

the chemicals. A larger number of rats per group and more dosage levels of the substance under study will aid in the interpretation of results and, therefore, reduce the possible risks of using a toxic substance or of missing a safe compound.

In some studies it is desirable to sacrifice one or two rats of each sex at various intervals, such as six, 12 and 18 months. If this is done, the total number in each group should not be reduced below 40 rats. Since the usual strain of albino rats is not inbred, weanling animals are assigned to the various groups according to litter mates. A litter of four males and four females, with a variation of no more than five grams within the litter, is distributed with one male and one female in each of the four groups as follows: (1) a control group, (2) a group on a low level which will produce no damage, (3) a group on a high level which approaches the tolerated amount and (4) a group in a middle level which may or may not produce damage. Rats are housed individually in an air-conditioned laboratory and records are kept of all observations for the duration of the experiment. If several animals are kept in a single cage, the possibility of observing individual variations is reduced and tissues of dead animals may be lost by their consumption by others in the cage; in general, this method of housing animals reduces the accuracy of the experiment.

In a similar experiment, dogs are used in groups of at least four each on the four dosage levels. Usually it is possible to select litters of young animals from a known breed, such as the beagle, and assign two males and two females to each group in a manner which will give the most even distribution according to age and weight. The suitable procedures for the dog study are discussed in detail in a previous section, on subacute toxicity.

During the whole experimental period the animal is considered as an individual, and observations are made of every change that affects the individual. These observations should include the rate of growth, food and water intake, general appearance, time of appearance and nature of any growth, behavior and mortality of the experimental animals. An animal showing significant loss of weight, the development of a large tumor, or other evidence of severe abnormality which may threaten its life during the experimental period should be sacrificed, autopsied for gross changes in the organs, and its tissues preserved for histopathological study. Tissues of animals dying within

the experimental period also should be preserved for study. Blood and biochemical studies are made during the experimental period. The importance of, and the usual procedure for, the latter are discussed in detail in previous sections of this article. Blood studies consisting of red cell count or hematocrit, hemoglobin determination, white cell count and differential are made at intervals of about three months on representative animals in each group. In the case of rats, usually ten animals from a group are sufficient, while in the case of the dogs, it is preferable to study all of them. Other blood studies, such as sedimentation rate, cell fragility and blood chemistry are done whenever the nature of the response so indicates.

The problem of dietary effects on the toxicity of chemicals is often raised. It is a finding in our laboratory, as well as in others, that the nature of the diet profoundly affects the results of a chronic-toxicity experiment. In the usual chronic-toxicity test, however, this problem has been simplified by the use of commercial diets which are prepared with the modern knowledge of animal nutrition. Unless a special item of a diet is under study to determine its effect on the relative toxicity of a chemical under varying conditions, a commercial diet is demonstrated to be nutritionally complete. Only experience with a diet will prove whether it is capable of producing optimal growth, longevity, etc., in control animals. With this in mind, it is more often advisable to select a proved commercial diet than to choose a so-called adequate synthetic diet which has not been shown to be adequate for long-term experiments. The adequacy of any selected diet will be shown by the response of the control animals, and these serve as a base line in each experiment. The chemical under study may affect the adequacy of a diet ("Agene," ethylene oxide). In these cases, nutritional studies are made to elucidate the nature of the harmful effect on the dietary constituents.

Upon completion of the feeding period, all surviving animals should be sacrificed and autopsied for gross changes in the organs, the principal organs should be weighed, and the tissues preserved for histopathological study. Researchers attach varying importance to organ weights. Some weigh nearly all of them, while others weigh only the liver and kidneys. We have found it practicable to weigh the liver, kidneys, spleen, heart, testes and, after fixing, the thyroid and adrenals. The latter two are weighed only when the chem-

ical is suspected of affecting these glands, or gross observation shows a change in size. Although the changes in liver and kidneys more often follow the histopathological changes in these organs, we have seen sufficient changes in the other organs mentioned for weighing to warrant the routine examination of their size. On the other hand, we rarely have seen significant changes in the weight of any other organ that relate it directly to the toxicity of the chemical.

A critical evaluation of all data on the effects of a compound—whether biochemical, pharmacological or morphological—will permit a logical decision to be reached regarding any possible hazard resulting from the use of the proposed substance. A substance proposed for use in foods should show no chronic toxicity to animals in an amount equivalent to 100 times the amount which is proposed for use in the total human dietary, that is, a safety factor of at least 100 should be present. In the necessary use of a chemical, such as a drug or a pesticide, this margin of safety may be reduced, and the recognized risk of its use may be weighed against its value to the human economy.

Occasions arise in which certain types of additions or modifications to the usual chronic-toxicity test become necessary. The following types of tests are suggested as aids in evaluating the hazards of some chemicals. They are not to be recommended for all proposed chemicals, and their value lies in their ability to detect particular responses of certain substances.

Reproduction Studies on Rats.—The inclusion of fertility studies in a chronic toxicity test may more than double the work to be done. The value of this increased work load must be measured against the need for such a test. The stresses of growth, pregnancy and lactation are all brought to bear on successive generations, and this added load may produce a significant increase in toxicity of a chemical. Although multigeneration studies have proved a severe test of the nutritional value of diets, we have found them to be a crude measurement in most chronic-toxicity experiments. They should be used whenever there is any indication that the proposed chemical may produce a selective response in the sex organs. The following outline illustrates how a reproduction study may be done:

Groups of 16 female and eight male weanling rats are placed on the control diet and on each of the experimental diets. When 100 days

old, they are mated. They are mated again ten days after weaning of the first litter (F 1a).

Sixteen females and eight males from the second litters (F 1b) are selected from each group at weaning and continued on their respective diets. When 100 days old, they are mated; then ten days after weaning the first litter (F 2a), they are mated again.

This procedure is repeated with the F 2b rats to produce the second litter (F 3b) of the third generation. When these are 21 days old, they are sacrificed, autopsied, and studied histologically.

In breeding, the male animals are rotated within each group weekly. Records are kept of mating, number of pregnancies, young in the litter at one, five and 21 days, and the weight of the litter at 21 days. If the litters are greater than ten in number, they are reduced to this number on the fifth day. Each group of rats is kept on its specific experimental diet throughout the test period. Parent generations are sacrificed and autopsied after the second litters are weaned, and young not selected for generation study are destroyed.

A compilation and analysis of all data will permit an evaluation of the accumulated effects of the chemical over several generations. Negative findings are encouraging but they do not show in themselves nontoxicity of the chemical.

Paired Feeding.—Paired feeding becomes important when administration of materials in a diet produces inanition in animals from an interference with their food consumption. Such substances have an unpleasant taste, poor texture, large bulk or other property which makes the new diet radically different from the animal's accustomed diet. In the paired-feeding experiment, at least ten pairs of litter-mate weanling rats of the same sex and approximate size are used for the control and experimental diets. One member of each pair is placed on the experimental diet and the other is placed on the control diet. The experimental dosage level selected from information gained in the subacute experiment is one which definitely produces inanition or other toxic manifestation. Food consumption is determined daily and the control animal is fed an amount of food equal to that which the experimental animal ate on the preceding day. In all other ways the paired-feeding animals are treated in a manner similar to those in the subacute experiment.

At the termination of the experiment, autopsies are performed, the principal organs are weighed, and tissues are preserved for microscopic examination. Growth curves are drawn for each animal, and the pairs are then compared. A statistical evaluation of the data will permit an interpretation of the cause and importance of the inanition.

VII. Carcinogenicity

by

Anne R. Bourke

That the possible carcinogenic activity of chemicals proposed for use in foods or in drugs must be considered as one manifestation of toxicity is well recognized by the toxicologist. He is acutely aware that such completely unacceptable pharmacological activity may exist in compounds which produce little or no systemic damage by prolonged feeding of relatively high concentrations.

Numerous factors influence the production of neoplasias in animals. Genetic predisposition to spontaneous tumors in the animal, time and duration of feeding of the carcinogen, the composition of the basal diet, and the suspending or diluting fluid used are but a few of the variables involved in the conduct of carcinogenic tests. It cannot be expected that the expert toxicologist will be completely at home in the field of the cancer-research expert. Conversely, the cancer-research expert is adequately occupied with the study of tumorous growth produced by chemicals and mode of action of compounds which can be relied upon to produce tumors under specified conditions. His primary interest is not broad screening studies to find new chemicals to produce the same tumors. Therefore, the detection of carcinogenic action of chemicals in human or animal diet falls more and more on the toxicologist.

In evaluation of the chronic-toxicity test, the number and types of tumors encountered are considered carefully. Results may indicate

a significant increase of tumors in animals fed test diets over those fed control. Carcinogenicity may also be suspected by reason of the compound's structural similarity to known carcinogen or substances reported to have produced tumors in animals. In the latter case the chronic-toxicity test may be modified to include a larger number of animals per level, and more than the usual number of levels of feeding at concentrations lower than those which might be expected to produce toxic symptoms. An experiment may also be designed whereby the chemical is administered by a route other than feeding, that is, inhalation, skin painting, implantation or subcutaneous inoculation. This is particularly desirable in the case of chemicals which might conceivably be used in drugs or cosmetics as well as in foods.

When completed, animal experiments can be interpreted to mean only that the test substance will (or will not) produce tumors in the particular strain of animals used when administered by the specified route within the time allowed in the test. It cannot be assumed that a chemical which induced tumors in mice by one route will also produce mouse tumors when administered by other routes, nor will it necessarily be tumorgenic for other animal species. Conversely, a chemical which shows no carcinogenic potency under one set of circumstances may be definitely implicated as a carcinogen when conditions are altered or a cocarcinogen is added. Accordingly, positive results in these animal tests can be taken as creating a suspicion that the chemical under study may be carcinogenic for man, but do not prove it to be so.

It is relatively easy to recognize potent carcinogens by their ability to produce a high percentage of neoplasms in several animal species under several experimental conditions. Such activity might well be apparent in the course of experiments designed for observations far afield from carcinogenesis. However, the complete study of conditions under which a chemical with very slight proclivity for tumor induction can be made to produce such tumors is an enormously expensive, complex and time-consuming procedure. Large numbers of several species must be administered the chemical by several routes, and observed over long periods of time. A workable compromise must be reached whereby danger of carcinogenicity will be detected when it is inherent in a chemical and of such potency as to constitute a hazard under the proposed conditions of use.

It is proposed that the routine chronic-toxicity test, wherein groups of one strain of rats are fed varying concentrations of the test substance throughout the lifetime, should be altered to include a larger number of animals in the control group. It should be supplemented by the feeding of the substance to one or more strains of inbred mice. Several factors require emphasis. First, since no colony of inbred rats in which the incidence of spontaneous tumors is well established and consistent is available commercially, the use of litter mates and relatively large numbers of rats is essential. Second, care should be exercised in the selection of strains and source of inbred mice. Information has been compiled as to sources of supply and characteristics of commercially available laboratory animals by the Institute of Animal Resources.[13] If it is decided that two strains of mice will be included in the test, one each of a known tumor-resistant strain and a tumor-susceptible strain should be used. Third, it is advisable to use a control group of animals equal in number to the total of all animals fed the test material. Evaluation of the potency of carcinogenicity of the material must be based entirely upon the gross and microscopic differences observed between the test and control animals as to per cent of animals developing tumors, number of tumors per animal, and time of appearance of tumors. Since some spontaneous development of tumors is genetically inherent in most strains of rodents, the number appearing in controls will serve as the base line. Fourth, all animals, tumor-bearing and tumor-free, should be submitted to autopsy. Microscopic pathological studies should be done on all tumor-bearing animals and on enough of the grossly tumor-free animals to assure the pathologist of the absence of minute neoplasia.

A special circumstance may occur in which routine screening testing of a substance for tumor-inducing activity may yield questionable or suggestive results. That is, the tumors appearing are too few to be statistically significant of induction by the test chemical, yet too many to be classified as of spontaneous origin. Resolution of such a question is complex in the extreme. It must be borne in mind that, in addition to the complete studies on rodents mentioned, it would be necessary to administer the substance to other species, which would probably include nonrodents, for a period of time approximating the lifetime of the animal.

[13] "A Handbook of Laboratory Animals" (National Academy of Sciences—National Research Council, Washington, D. C., 1954, Publication 317).

VIII. Dermal Toxicity

by

John H. Draize

Substances applied topically to the skin or mucous membranes may elicit (1) local effects (local toxicity) and (2) systemic effects (systemic toxicity). The local effects are more properly termed "irritations," a general term to describe essentially eczematous or contact dermatoses, in case of the skin, or of inflammation, in case of the mucous membranes. Skin irritation may result from skin contact with substances which are primary irritants or from contact with substances producing sensitizations. Recently, however, a third type of skin irritation has been recognized which, for the lack of a better term, has been named skin fatigue.

Local Toxicity—**Primary irritation of skin.**—A primary irritant is an agent which will produce a dermatitis by direct action on the normal skin at the site of contact if it is permitted to act, in sufficient concentration, for a sufficient length of time. The resulting dermatitis elicited by the primary irritant will depend on the agent's inherent nature, its concentration, and the total elapsed time of exposure.

Primary irritation of the skin is measured by a patch-test technique on the abraded and intact skin of the albino rabbit. The method [14] consists in introducing under a patch 0.5 ml. (in case of liquids) or 0.5 grams (in case of solids and semisolids) of the test substance. The animals are immobilized in an animal holder with patches secured in place by strips of adhesive tape. The entire trunk of the animal is then wrapped in rubberized cloth for the entire 24-hour period of exposure. This latter procedure helps hold the patches in position and, in addition, retards the evaporation of volatile substances. After 24 hours of exposure the patches are removed, and the resulting reactions are evaluated on the basis of scores (Table 3).

[14] Draize, Woodard and Calvery, work cited at footnote 3.

Table 3

Evaluation of Skin Reactions

(1) Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
(2) Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm.)	3
Severe edema (raised more than 1 mm. and extending beyond area of exposure)	4
Total possible edema score	4

Readings are made also after 72 hours, and the final score represents an average of the 24- and 72-hour readings. An equal number of exposures are made on areas of skin which have been previously abraded. The abrasions are minor incisions through the *stratum corneum*, but not sufficiently deep to reach the derma (that is, not sufficiently deep to produce bleeding).

The total erythema and edema scores are added in both the 24- and 72-hour readings, and the averages of the scores for intact and abraded skin are combined; this combined average is referred to as the primary irritation index. It is useful for placing compounds in general groups with reference to irritant properties.

Compounds producing combined averages (primary irritation indexes) of 2 or less are only mildly irritating, whereas those with indexes from 2 to 5 are moderate irritants, and those with scores above 6 are considered severe irritants.

Primary irritation of mucous membrane.—Irritation of mucous membrane is measured on the rabbit eye and penile mucosa. In the case of the penile mucosa the preparation is applied so that thorough wetting is attained. Approximately 0.2 ml. of the preparation is required. The erythema and edema are evaluated on the basis of scores which total up to 4 for the severe injuries, that is, the varying degrees of erythema and edema may each have a maximal score of 2. If a

preparation is found sufficiently irritating to cause necrosis and sloughing of the mucosa, the agent is reapplied in sufficient dilution so that no necrosis or sloughing results. Reactions are read at one, two, 24 and 48 hours after application.

In the technique of tests of toxicity of substances to eye mucosa,[15] observation of injuries are made on the cornea, iris, and the bulbar and palpebral conjunctivae. Numerical scores are assigned to the lesions observed. In this system of scoring, the injuries to the cornea and iris may account for approximately 80 per cent of the score; these structures are purposely weighted because of their vital role in vision.

Albino rabbits are used for eye-mucosa toxicity studies. One tenth of a milliliter of the substance is instilled in one eye; the other eye, remaining untreated, serves as control. A series of nine albino rabbits are used for the testing of one substance. In the case of the first three subjects, the treated eyes remain unwashed. Since washing the eye may or may not alleviate symptoms of injury, the six remaining subjects are divided into two equal groups. In the first of these two groups, the eyes are instilled with the test substance and washed with 20 ml. of lukewarm water (approximately at body temperature) two seconds after instillation of the test substance. In the second group, the treated eyes are washed with a similar amount of lukewarm water four seconds after instillation of the test substance. The washing procedures are regarded as significant, since it is important to know the effect of such a procedure—whether it is beneficial or detrimental and, if beneficial, the extent of the benefit.

Ocular reactions are read either with the unaided eye or with the aid of a hand slit lamp. Readings are made at 24, 48 and 72 hours, and at four and seven days after treatment, or as long as the injury persists. A preparation which has elicited corneal and iris lesions which have not cleared by the seventh day is considered a severe eye irritant. The cornea is scored on the basis of the density of the opacity and the total area involved. The iris is scored on the intensity or degree of inflammation exhibited, and the palpebral and bulbar mucosae are scored on the extent of the chemosis, redness and discharge.

In this system such serious corneal lesions as pannus and phlyctepar reactions are not given a numerical score. Any preparation eliciting

[15] J. H. Draize and E. A. Kelley, "Toxicity to Eye Mucosa of Certain Cosmetic Preparations Containing Surface Active Agents," 17 *Proceedings of the Scientific Section of Toilet Goods Association* 1 (1952).

such reactions is deemed to be too severely irritating for use about the eyes. Table 4 contains a scale for scoring ocular lesions.

Sensitization studies—GUINEA-PIG TECHNIQUE.—A cutaneous sensitizer is an agent which does not necessarily produce demonstrable

Table 4

Scale for Scoring Ocular Lesions

(1) Cornea	
(A) Opacity—degree of density (area most dense taken for reading)	
No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
(B) Area of cornea involved	
One quarter (or less), but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
A × B × 5 Total Maximum = 80	
(2) Iris	
(A) Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
A × 5 Total Maximum = 10	
(3) Conjunctivae	
(A) Redness (refers to palpebral conjunctivae only)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
(B) Chemosis	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
(C) Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score (A + B + C) × 2 Total Maximum = 20	

The total score for the eye is the sum of all scores obtained for the cornea, iris and conjunctivae.

changes on first exposure but may effect tissue changes after a number of exposures, producing a dermatitis at the point of contact or in other parts of the body.

Various techniques have been developed for the determination of potential cutaneous sensitization. Landsteiner and Jacobs [16] have demonstrated that a guinea-pig intracutaneous method was adequate to detect cutaneous sensitizers among relatively simple chemical compounds. This method was modified slightly [17] and has been used generally by this laboratory.

White male guinea pigs weighing 300-450 grams and subsisting on a commercial rabbit pellet ration supplemented with greens (kale or lettuce) are identified, and hair removed from back and flanks by close clipping. A 0.1 per cent solution or suspension in physiological saline of the material to be tested is injected intracutaneously, using a 26-gauge hypodermic needle. The injections are made every other day, or three times weekly, until a total of ten have been made. The ten sensitizing injections are made at random in an area of the back and upper flanks. This area measures three or four centimeters square. The retest injection is made in an area just below the region or sites of the sensitizing injections. The first injection consists of 0.05 ml., while the remaining nine injections consist of 0.1 ml. each. Two weeks after the tenth injection, a retest injection is made, using 0.05 ml. of a freshly prepared solution or suspension as before. Twenty-four hours following injections, readings are made of the diameter, height, and color of reaction. A comparison of the reaction following retest is made with the average of the readings taken after each of the original ten injections. If the value for the retest reading is substantially higher than for the average of the ten original readings, the substance can be said to have produced sensitization. The degree of sensitization is proportional to the increase in the final reading compared to the average of the readings following the ten original doses.

SENSITIZATION TECHNIQUE IN MAN.—The guinea-pig intracutaneous technique should be used to screen all materials to obviate exposing man to severely sensitizing substances. In the case of compounds which may be only mildly sensitizing or in which the guinea-pig method is unable to furnish unequivocal results, man appears to

[16] K. Landsteiner and J. Jacobs, "Studies on Sensitizations of Animals with Simple Chemical Compounds," 61 *Journal of Experimental Medicine* 643 (1935).

[17] Draize, Woodard and Calvery, work cited at footnote 3.

be the only feasible test subject. These human tests should employ preferably 200 individuals (100 male and 100 female, covering as wide an age range as possible). The test material, 0.5 ml. (or 0.5 gms. if solid), is applied by patch to an area (randomized) on the arms or back. The patch is removed after 24 hours, and a reading of the reaction is made. The area of erythema and edema is measured. The edema is estimated by the elevation of the skin with respect to the contour of the unaffected normal skin. The subjects are given a day's rest and then given a second such patch application. This procedure is repeated until a series of ten consecutive exposures have been made. After this series of ten applications, the subjects are given ten to 14 days of rest, after which a challenge or retest dose is applied once; procedure for retest application is similar to any one of the applications of the original ten exposures.

A comparison of the reactions observed during the ten sensitizing doses with the reaction obtained following the retest dose permits an estimation of the degree of sensitization (if any) obtained.

Repeated patch applications of substances to both man and animals reveal that certain substances which at a given concentration do not produce primary irritation may elicit severe skin reactions after a number of exposures. Nevertheless, these reactions may not be considered incidents of sensitization, since after the ten to 14 days of rest the skin recovers its original resistance to injury by the substance. Such substances are neither true primary irritants nor sensitizers. For the lack of a better term these reactions have been identified as "skin fatigue." A number of chlorinated compounds have, in our experience, produced this type of reaction.

Compounds eliciting this reaction may be detected by the repeated patch technique outlined above.

Systemic Dermal Toxicity.—Appraisal of the systemic toxicity is made following dermal applications by acute (single) and subacute (multiple) exposures to the test substance. It includes observation of symptomatology, chemical studies, hematology, pathology and physiological function tests on treated subjects.

Acute dermal toxicity (single exposure).—In the acute exposures the agent is held in contact with the skin by means of a rubber sleeve [18] for periods varying up to 24 hours. The sleeve, made of rubber dam, is so constructed that the ends are reinforced with additional strips

[18] Draize, Woodard and Calvery, work cited at footnote 3.

of dam and, over-all, should fit snugly around the trunk of the animal. The ends of the sleeve are tucked, permitting the central portion to "balloon" and furnish a reservoir for the dose. The reservoir must have sufficient capacity to contain the dose without pressure. In Table 5 are given the dimensions of sleeves for several species of animals and the approximate body surface exposed to the test substance. The sleeves may vary to accommodate smaller or larger subjects of a given species.

Prior to exposure the animals are prepared by clipping the skin of the trunk free of hair. Approximately half of the animals are further prepared by making epidermal abrasions every two to three centimeters over the area of exposure. The abrasions are sufficiently deep to penetrate the *stratum corneum* (horny layer of epidermis), but not to disturb the derma (that is, not to obtain bleeding). In the case of dry powder preparations, the skin and substance are moistened with physiological saline prior to exposure.

The sleeve is slipped onto the animal, which is then placed in a comfortable but immobilized position in a multiple animal holder.[19] The doses of liquids and solutions are calculated on the basis of body weight and introduced under the sleeve. In the case of liquids or solutions of unknown toxicity, preliminary dose ranges of 3.9, 6.0 and 9.4 ml./kg. are employed in rabbit exposures. Continuous exposures to the agent never exceed 24 hours when such exposures require some degree of immobilization. If there is slight leakage from the sleeve, which may occur during the first few hours of exposure, it is collected in a glass receptacle and reapplied. A sufficient number of animals at different dosage levels are used that it will be possible to calculate the dose which will be fatal to a given percentage of the animals. This can be determined from mortality ratios obtained at various doses employed. The dose which gives a 50 per cent mortality (LD50) is the one usually calculated. An analysis of the data enables one to determine the slope of the dosage-response curve, as well as the standard error in the determination.

In the testing of materials with unctuous characteristics which adhere readily to the skin, a 20-mesh wire screen is usually employed instead of the rubber sleeve. The screen is padded and raised approximately 2 cms. from the exposed skin. It is constructed in varying sizes to fit various animal species, as is the case with the rubber sleeves.

[19] Draize, Woodard and Calvery, work cited at footnote 3.

The animals are prepared in the same manner as for sleeve exposures. In cases of finely divided powders, the measured dose is evenly distributed on cotton gauze, which is then secured to the area of exposure. The rubber sleeve or screen is then slipped over the gauze which holds the dose applied to the skin.

At the end of the period of exposure, the sleeves or screens are removed, the volume of unabsorbed material—if any—is measured, and the skin reactions are noted. The subjects are observed for gross symptoms of poisoning, and are transferred to metabolism cages where they are observed for a minimal period of two weeks. Body weights, food consumption, behavior, etc., are checked daily. Urine is checked for the presence of protein, reducing substances, blood pigments, or any abnormality in gross appearance. Observations for changes in blood morphology are made. The tissues of animals which die several days after exposure and those which are sacrificed because of severe poisoning are examined for histopathology.

Subacute dermal toxicity—20-DAY EXPERIMENTS.—(1) Topical applications: In the 20-day subacute experiments, relatively large doses of the substance are applied daily. Applications are made by inunction to an uncovered area of intact and abraded skin. Preparation of the skin is similar to that prior to the acute exposures. The size of the dose employed depends upon the data obtained in the acute exposures, but several levels are employed, with the top dose level rarely exceeding 6.0 ml./kg. The area inuncted is approximately 10 per cent of the total body surface of the animal. The material is applied by gentle inunction, using a glass rod, which involves manipulation of the hair shafts and mild pressure. The animals are observed

Table 5

Dimensions of Sleeves for Various Small Laboratory Animals

	Measurement in Centimeters		*Range of Weight of Animals (grams)*	*Average Area of Exposure cm2*	*Percentage of Total Body Surface*
	Diameter at Ends	*Over-all Length*			
Mouse	2.5	4.5	20–35	9	8.6 *
Rat	4.5	9.0	200–350	90	23.4
Guinea pig	6.0	10.5	500–750	90	14.5
Rabbit	7.0	12.5	2,500–3,500	240	10.7
Dog	9.0	12.5	4,000–6,000	500	16.0

* The values in this column are for animals in the middle of the body weight ranges given. The calculations were made by Meeh's formula (G. Lusk, *The Science of Nutrition* (4th Ed., W. B. Saunders Company, Philadelphia, 1928), p. 122.

for a two-week period following the final exposures, and data on mortality ratio are treated statistically as in the case of the acute dermal-toxicity experiment. In the subacute experiments, the daily repetition of the doses for three weeks and over-all observation for a five-week period yield valuable information on the action of agents on the skin (local toxicity), as well as their effects on remote tissues (progressive systemic effects). In some cases there is a progressive deterioration of the skin, so that it becomes a less effective barrier as doses are repeated. In other cases the agent may produce a mummification or a coagulative effect on the skin or severe eschar formation, so that absorption is retarded.

The same observations and tests on the subjects are made as in the acute dermal experiments. The urine and blood are so examined, and microscopic examinations are made of the tissues of some animals.

(2) Impregnated clothing: Certain substances—medicaments, insect repellants, lousicides—may be impregnated in wearing apparel. Exposures to such impregnated wearing apparel may not be without danger. In the appraisal of such dangers, suitable measured strips of an absorbent fabric are impregnated and secured around the body trunk of the test animal. Herringbone cotton twill (8½ oz. yd.) has been found suitable material for this purpose. Strips are accurately measured as to surface area and the impregnant incorporated in the cloth at a measured weight or volume of the substance per square foot of cloth. Since many impregnants are solid or semisolid materials, it is convenient to dissolve the given dose in a volatile solvent such as acetone, using a sufficient quantity of solvent to allow complete and uniform impregnation of the cloth. The solvent is blown off by suspending the cloth in a gentle current of air. The cloth thus impregnated is secured around the body trunk of the test subject and allowed to be worn undisturbed for a period of three days. The cloth is then removed, laundered and reimpregnated as above and immediately reapplied to the animal to be worn for the balance of the week. This procedure is repeated during the second and third weeks of the experiment. During the three weeks of experiment the animals are exposed to a total of six impregnations.

Impregnation levels employed will depend upon the proposed manner of use, but quantities eight- to ten-fold that proposed for use should not prove deleterious in these animal experiments to demon-

strate an adequate margin of safety. The exposures should include subjects with abraded and intact skin.

This technique may also be employed to assess inherent dangers to workers whose wearing apparel may become contaminated with toxic sprays or other materials during the course of their normal work.

The same observations and tests are made, and the data treated statistically, as in the section "Subacute dermal toxicity—20-day experiments—(1) Topical applications," above.

90-DAY EXPERIMENTS.—Although the 90-day dermal-toxicity experiments involve greater detail and are more time-consuming, these procedures yield the most information on the potential local and systemic effects which may be exerted by an agent. A minimum of four dose levels is employed. The lowest dose level (so-called base line dose) is calculated as the maximal amount to which the individual may become exposed in the course of use as per label directions or from such use as is customary or usual. The size of the three other levels of dosage is obtained by multiplying "base line" dose by the factors 2, 4 and 8, respectively. In case it is not possible to ascertain what quantities may actually be used in practice, arbitrary dose levels of 0.5, 1.0, 2.0 and 4.0 ml. per kilogram of body weight are employed. Doses are applied daily for a 90-day period by inunction to the clipped *intact* skin of the animals. The areas inuncted are measured so that approximately 10 per cent of the entire body surface is treated. The experiment is similar in procedure to the 20-day subacute-toxicity experiment, except that the longer period of treatment with lower dosage levels permits the study of agents which may produce progressive deterioration in the animal. The same observations and tests are made as in the shorter subacute experiment. Special emphasis is placed on blood morphology to ascertain possible effects of the agent on the hemopoietic organs or the blood itself. If there have been any observable effects, the tissues of *all* the animals, regardless of behavior during the experiment, are examined for histopathologic changes.

Compounds which may be but slightly toxic as judged by acute dermal exposures may be severely damaging upon repeated exposure (20- or 90-day). Compounds which behave in this manner often do not cause gross skin changes at first exposure, but produce severe skin changes upon repeated exposures. The skin injuries may range from a mild dermatitis to complete necrosis or severe eschar formation

with desquamation. The regenerated skin, as a rule, is more resistant to future action of the compound.

The rate of penetration of a compound into the skin, and local toxicity (local skin effects) noted following single and multiple exposures, comprise information equally as valuable as the information on the systemic effects obtained in these dermal-toxicity experiments.

From the data obtained by the procedures as outlined to assess local and systemic toxicity of substances applied topically to skin and mucous membranes, an appraisal or evaluation of extent of injury may be made. It should yield, also, information on prognosis for repair and recovery. Assessment of data, a consideration of species differences, and the allowance for a margin of safety, should make possible an estimation, in the case of a given compound, of the amounts, concentration, and frequency of application that may be tolerated by man.

IX. Pathology

by

Arthur A. Nelson, M. D.

This section differs from that in the 1949 "edition" of this review principally by the addition of comments based on numerous discussions of mutual problems with university and industrial scientists, and by references to drugs and cosmetics. However, as might be expected, the general principles have not changed. It is true that recent developments have placed added emphasis on such factors in toxicity tests as radioactive isotopes, metabolic studies and carcinogenic potency. Of these, the last has the pathologist's greatest concern, and he shares in the planning of appropriate studies with biochemists, statisticians, and others. Nevertheless, the crucial aspect of the pathologist's job in this respect is still the same as in former years, namely, to be able to say whether there is or is not a tumor present and, if present, of what type, and what might be its relation to the treatment.

General Considerations.—"Pathology" as used in this section means primarily gross and microscopic morphological pathology—in other words, pathological anatomy and histology, and not functional pathology.

Since the ideal cannot ordinarily be attained easily or without undue expense, practical considerations dictate that the type and amount of pathological study be related to such factors as the present and possible future uses of the chemical in question (for example, whether in a food, drug or cosmetic), its level of incorporation, its known degree of toxicity, its chemical relation to known human toxicants, the total human intake by all routes, the possible duration of human intake, etc.

In the case of a food, such as bread or milk, which forms one of the major parts of the average diet, and which might be consumed over a large part of the lifetime of an individual, detailed pathological studies on several species are a necessity. By detailed study is meant autopsy and thorough gross examination of all experimental and control animals, and microscopic examination of all major organs and tissues, in sufficient numbers to make reasonably certain whether or not the chemical is causing injury or change in any of them. If there is any effect, the examination should show the lowest dosage level of the chemical capable of producing it in even the slightest degree. More will be said later about the term "slightest." Simple though the concept be, it is not enough realized that it is considerably more difficult to determine the lowest level at which a minimal effect occurs than it is to determine in a general way that the given chemical has such-and-such a degree of toxicity.

As the relative importance and potential hazard of a given chemical to the public decreases—for example because of less frequent intake as with a drug or less total intake in a minor, as opposed to a major, food—pathological studies could reasonably be lessened in about the same ratio as would animal feeding, biochemical studies, etc. The variables here are so numerous that nothing specific can be stated. Generally speaking, it will amount to fewer animals, of fewer species, being microscopically examined in detail. As a minimum, however, detailed microscopic (and, of course, gross) examination of enough animals to give significance to the results would still be a necessity.

Organs and Tissues Examined.—The practice of sending a pathologist small fixed portions of various organs after a relatively untrained person

has performed more or less of an autopsy is to be condemned. So is the sending of a piece of abnormal tissue only, without an adequate amount of adjacent uninvolved tissue. The same pathologist who examines the microscopic slides should, if possible, either perform or watch the autopsy, or should arrange with a qualified person to obtain detailed and accurate data therefrom. Every gross lesion, external and internal, should be described and, if possible, measured, and not merely stated as, for example, "subcutaneous tumor" (of unspecified size, location, consistency and appearance) or "nodular liver" (of unspecified size, color, consistency, shape, degree of nodularity, and approximate size of nodules). With due allowance for viewing some animals in the fresh condition and for some taking of organ slices for special fixatives, it may be convenient to send the fixed viscera of rats and smaller animals en bloc to the pathologist; organ weights are not significantly affected for comparative purposes, and the better gross examination in our opinion outweighs the possible objection of less good fixation (and the handling and slicing of these small organs before fixing introduces its own set of artefacts).

A list of the organs usually examined in our dogs may be of some help as a guide. These include lung, heart, liver, gall bladder, spleen, pancreas, mesenteric and cervical lymph nodes, stomach (fundic and pyloric), small intestine (three levels), colon, kidney, adrenal, urinary bladder, testis (or ovary), prostate (or uterus), thyroid, parathyroid, submaxillary salivary gland, brain (four levels), hypophysis, rib bone and bone marrow, skeletal muscle, and any structure not mentioned which was found abnormal on gross examination. Some of these are omitted if a number of dogs given the same substance have been examined in the more detailed manner and the organs in question found to be normal. On the other hand, routes of administration other than oral will call for the sectioning of additional structures, such as skin, local injection site, etc. Rabbits and smaller animals get an examination slightly less detailed than do the dogs. The foregoing remarks apply to chronic experiments; in pilot and subacute studies on the smaller animals (assuming that a chronic experiment is also to be done), microscopic examination may be considerably abbreviated.

Supplementary tissue fixatives and staining techniques, in addition to the routine ones, generally will be required, to at least a small degree. Even when not absolutely required, they are very helpful in settling doubts that arise when reliance is placed on only a single

routine type of section. Examples of the more useful special stains ("useful" in the sense of greatest help per man-hour expended, and "stain" not calling up the technical nuances of whether it is a stain, an impregnation or a reaction) are those for the identification of pigments, for fat in frozen sections, for Giemsa-type staining of imprints or smears of bone marrow, and the periodic acid-Schiff technique for glycogen, reticulum, and general surveying. We have found routine staining of liver and kidney for fat, in animals of rabbit- and larger-size, to be decidedly helpful. So has been a permanently set up apparatus for examination by polarized light.

Thorough pathological examination of the nervous system is such a complex and specialized field that nothing beyond the routine type of examination as done by the general pathologist is discussed in this section. Our experience has been that chronic-toxicity experiments will on occasion give rise to specific brain changes, but that more often any brain lesions present will either be clearly incidental or else of the incidental type but with a possible predisposing factor in the treatment. The hypophysis, even though it be the "master gland," is not usually examined in detail, partly for reasons similar to those for the brain. In any event, if the brain is taken out, the hypophysis should also be taken and at least a routine section made.

Newer developments in histochemistry raise the question of whether or not some of these techniques applied to tissue sections might give more satisfactory answers regarding pathological changes than do the more common staining methods. Obviously, by their very nature they give some answers that the other techniques cannot give. Usually, more time and effort per finished preparation is required, and the degree of permanency is less. Generally speaking, histochemical studies should be more useful in short-term toxicity studies where the relative stress is greater and where alterations of chemical content in a cell or organ are better shown than they would be in long-term studies with their predominantly structural alterations.

Only in comparatively recent years has it become generally realized how many deviations from the ideal "normal" miscroscopic appearance of the organs of laboratory animals can be caused by even slightly suboptimal diets, how many changes induced during experimental procedures are simply the nonspecific ones of inanition, and how many "spontaneous" pathological changes may be found in con-

trol animals, especially older ones. Therefore, without adequate control material a pathologist must (or at least should) carefully consider every other possibility than a specific effect of treatment when he sees a gross or microscopic anatomical deviation from the ideal "normal." Under the circumstances stated, it is often difficult to make the differentiation. If only obvious abnormalities in uncontrolled experimental animals are to be considered, much may be missed.

With the larger animals, such as dogs and monkeys, the expense of procurement and maintenance makes for serious consideration of every animal added to an experimental group. On the other hand, too few animals per dosage level may easily lead to equivocal or erroneous results. For example, it is commonly proposed by other laboratories that a chemical be tested for its chronic toxicity in dogs by feeding it at two levels to groups of two dogs each, with another group of two as controls. With such a scheme there are two main sources of trouble. One is that the compound may cause significant lesions in, say, only 25 per cent of the subjects at the given dosage level. Therefore, if only two, three, or even four dogs are used at that dosage level, there is a good chance that the group will not include a susceptible animal. The other source of trouble is that in such a group it is quite likely that some incidental lesion will occur, perhaps of such nature that assignment as between treatment and chance is difficult. From the pathological standpoint of trying to avoid these troublesome factors and yet keep the dog numbers at a minimum, we have found the smallest reasonably satisfactory number to be four per group, assuming an adequate number of dosage levels.

Tissue alterations resulting from the administration of chemicals are frequently not of a new or characteristic type; rather, there is often but an increase in the incidence or degree of some type of lesion already present in that particular strain of animal. This is particularly true for the two-year or "lifetime" experiments in rodents, in which a low-grade chronic stress may not begin to give significant differences between test and control animals until during the second year. For example, older rats on control diets may show a relatively slight degree or incidence of a chronic nephritis or nephrosis. Those fed the test diet may show the same histological changes in the kidney, but the process will, on the average, be of greater intensity or frequency, or both. If such a difference is beyond the reasonable bounds of chance, it should be considered an effect of the chemical.

The same applies to significant differences in tumor incidence, either in a particular organ or with respect to tumors in general, which may not assert themselves until in the last half of the second year. When more specific effects, such as hemorrhage or massive liver necrosis, are observed, there is of course little doubt as to the agency responsible. However, this frequent occurrence of some amount in the controls of what is present in greater amount in the treated animals is a major factor in necessitating careful tabulation of all pathological changes in an experimental series of animals. Because disease conditions in a laboratory colony of animals will fluctuate from year to year, control animals should approach the ideal of litter mates of the same sex and weight as nearly as possible, and in any event should be run concurrently with the test animals.

Control animals in experiments involving repeated subcutaneous injections, stomach tubing, and so on, should not be merely untreated animals, but should be given injections and tubings as similar as possible to the test group. Even then, differences that can seriously affect the results may occur—for example, the greater retention of heated, than of unheated, fats at the site of subcutaneous injection.

An excellent stimulus to objectivity in examination, particularly when effects of treatment are relatively slight, is for the pathologist to receive the animals under some code designation, at least until a preliminary report has been made. For instance, he might be told that Rat 8888 of Group C had been found dead after 78 weeks of the experimental period had elapsed, but he would not know whether the group that the rat belonged to was one of the four treated groups or the control group. Group identification is important, however, because slight effects of treatment can be much more easily seen on concurrent comparison of several members each of two or more groups than on study of isolated specimens, separated in time. Particularly in evaluating minimal degrees of microscopic liver changes, we have found the coding principle helpful simply because it eliminates the mental effort of denying the knowledge of dosage level a place in the evaluation of the histological changes.

Evaluation of Histological Changes.—The evaluation of the microscopic pathology demands a "fine cut," rather than a coarse, approach. It is not enough that the slides from the control animals be looked

at, then those from the treated animals, and then a mental comparison made and recorded. Such a procedure would be suitable if only pronounced changes were being sought, but it must be stressed that it is not suitable when the very slightest effects, those at that level of feeding at which the chemical begins to have any action whatever that changes the normal, are the effects being sought. The grosser effects at the higher levels of dosage, whose determination is equally necessary in order to give the "spectrum" of the chemical's toxicity, will be easy enough to delineate.

The "fine cut" approach requires detailed tabulation, particularly for those organs more commonly showing changes. For instance, in studies of certain chlorinated insecticides the following items were graded in the liver sections: characteristic rodent "chlorinated insecticide" change, hepatic cell vacuolation, bile-duct proliferation, average hepatic cell size, irregularity of hepatic cell size, disorganization of normal lobular pattern, and foci of small hepatic cells. No individual microscopic slide should be without some sort of notation concerning it, and preferably in tabular form. Such tabulations need not be especially fancy or pretty in their physical form, but they contain the "raw" individual data from and upon which are built the later deductions and summarizations, and they should be of research quality.

X. Statistics

by

Bert J. Vos, M. D.

Statisticians frequently remind us that their services are used to best advantage if their advice is sought before the start of an experiment instead of after its completion. The fact that the present section follows all the others should be taken as evidence that this point still

bears repeating. The correct planning of a toxicological study must rest on a sound statistical basis as well as on a thorough familiarity with the characteristics of experimental animals in their response to various materials. The experimental designs which have been proposed in the various preceding sections should not be taken as rigid directions, but rather as starting points from which the toxicologist can set up experiments which will fit the peculiar needs of the problem under attack. An investigator who follows a stereotyped set of directions may make poor use of the experimental material at his disposal.

The question of "how many animals" frequently arises during the planning of a toxicity study. Since each additional animal, if properly used, gives us additional information about the compound under study, the question can properly be turned back to the inquirer in the form: "How much information do you want?" To the extent that the answer to this latter question is vague or extravagant, so also must be the answer to the original question. The fact that information increases only in proportion to the square root of the number of animals used rather than directly in proportion to the number serves to discourage expansive projects. Frequently the number of animals finally decided upon represents a compromise between what we want to know and what we can afford to find out.

In an acute-toxicity test, the question of "how many" must be resolved as the experiment progresses. This is so because the accuracy with which the LD50 is determined depends on the slope of the dose-response curve as well as on the number of animals used. If the compound being tested is found to have a steep slope, fewer animals will be needed to determine the LD50 with a given precision than if the slope is relatively flat. Fortunately, the very nature of acute-toxicity tests permits one, with little inconvenience, to use additional animals until the desired precision has been reached or until it becomes apparent that the slope of the dose-response curve is so flat that such precision is unpractical. In the case of the more costly animals, such as the guinea pig, rabbit, cat or dog, one is usually content with a very rough approximation of the LD50.

In the subacute and chronic experiments, arriving at the correct number of animals is more difficult, for two reasons. In the first place, because of the longer duration of these experiments, it is seldom

feasible to add more animals as the need becomes apparent. It is generally a case of repeating the entire experiment. In the second place, these longer experiments have as one of their objectives the determination of the dosage level at which the first minimal toxic effects occur. These effects will be found only in the more sensitive animals and the larger the group the greater the likelihood that these sensitive animals will be included. As an example of the number of animals that may be required to detect small effects, it may be stated that an effect which occurs in 1 per cent of the test animals will be entirely missed more than one third (37 per cent) of the time, if only 100 animals are used. If the effect is one which occurs spontaneously in the control animals and we are concerned with detecting a 1 per cent increase in incidence in the treated animals, the task is even more formidable. Yet if the effect were to occur in 1 per cent of the population of a country the size of the United States, it would represent an impressive total. Fortunately, the behavior of the compound at the higher dosage levels in the experimental animals is of considerable assistance in interpreting the findings at the lowest dosage level. Essentially, one makes use of a rough dose-response curve similar to that used in the acute-toxicity tests.

Various strains of the same species may differ considerably in their susceptibility to toxic materials. Even the susceptibility of a single strain in one laboratory may change from year to year. If there were some way in which this susceptibility could be measured it would be most helpful in evaluating the results obtained on a new compound being studied. It has been suggested that a group of animals receiving a related known toxicant—a so-called positive control group—be included in each experiment in an attempt to solve this difficulty. Thus, in a test on a new chlorinated hydrocarbon insecticide, one group of animals would receive DDT for purposes of comparison. If in this experiment the DDT appeared less toxic than usual, one would need to be more conservative in evaluating the results on the new insecticide. The weakness of this approach is that it assumes a correlation in the response of animals to the two substances. In the field of bioassay it has been adequately shown that a close similarity in chemical structure is no assurance of similarity in response to changing sensitivity in the test animal. Ouabain, for example, proved to be a poor reference standard against which to assay digitalis.

We are not familiar with any really satisfactory statistical procedure for making significance tests on growth curves. Our usual practice is to plot the data on graph paper and by inspection form an impression as to which dosage levels result in growth significantly different from that of the controls. Where the difference from the controls is slight, some help can be got by selecting a date during the rapid-growing period and making a significance test of the difference between the average weights of the control animals and those receiving the dosage level in question. For this purpose, either the "t" test or the analysis of variance may be employed, depending upon which is more familiar to the experimenter. If the experiment was set up using litter mates, advantage should be taken of this fact by testing whether the average of the differences in the respective pairs differ significantly from zero. At this point a word of caution should be expressed with regard to the possible growth-stimulating effects sometimes observed at one or more of the low dosages of the test substance. When such an event is encountered there is often a temptation to disbelieve it and assume that the control group is for some reason underweight. If, on the basis of such an assumption, these growth-stimulated animals are averaged in with the controls, or worse still, are used in their stead, a false conclusion may be reached as to which of the remaining doses of the test material has inhibited growth.

In the evaluation of the results of biological experiments, the method of the statistical analysis of the data is fixed by the design of the experiment. The proper design and subsequent analysis permits the maximum amount of information to be extracted from the data. Essentially, the statistical analysis compares the effect due to differences in treatment with the proper estimate of the experimental error. The probability may then be calculated that the observed results are due to the differences in treatment as opposed to chance variation.

In summary it may be said that the procedures which have been outlined in the preceding nine sections represent a working compromise regarding number of animals and detail with which they are studied. They have been designed to give the most efficient use of experimental animals and investigator's time under the conditions of our laboratory. Those working under other conditions may well find that a different emphasis better serves their particular needs.

XI. Interpretation of Animal Toxicity in Terms of Potential Toxicity in Man

by

A. J. Lehman, M. D., and Geoffrey Woodard

Dr. A. L. Tatum, emeritus professor of pharmacology at the University of Wisconsin, said:

> People are rather unpredictable and don't always die when they are supposed to, and don't always recover when they should. All in all, we must depend heavily on laboratory experimentation for sound and controllable basic principles.

The experimental approach which is necessary for developing the basic principles is dependent to a considerable degree on the objectives desired by those interested in the potential toxicity of a chemical for man. Based upon our own experience, these objectives may be grouped into those desired by the *clinician*, the *public-health official* and the *industrial toxicologist*.

The *clinician* is interested in knowing the dosage of the chemical that may be given safely to his patient. He wishes to know what side effects of the drug he should be on the lookout for. He would like to have an understanding of the pharmacodynamic actions of the drug so that he may have proper antidotal measures prepared in the event of an overdosage. He is also interested in knowing what other drugs may be used safely in combination with this one. Conversely, the clinician wants to avoid the use of other drugs that are potentiated by this one. Finally, he would like to give the drug in a sufficient dosage and at the proper intervals of time to provide adequate blood or tissue levels for effective therapy.

The *public-health official* is interested in the potential toxicity of a chemical that will be used in pest control, sanitation, preservation or food-processing. He is mostly concerned with the long-continued contact of small amounts of the chemical on man or in foodstuffs. He wishes to know what quantity of the chemical may be ingested, absorbed or inhaled safely over very long periods of time without causing perceptible damage to man. Some specific objectives are to avoid the widespread use of chemicals that may be carcinogenic or produce sensitization, and to be certain that the amounts of the chemical that will be used will not, even in susceptible individuals, produce injury to important organs or systems of the body. He also must face realistically the many problems that have arisen through modern food-processing, packaging and distributing practices.

The *industrial toxicologist* has two primary objectives. It is his duty to protect plant personnel from hazardous dusts and gases, and he must be certain that the products distributed by his company can be safely handled in commercial channels. Since the end use of the chemical cannot always be controlled or anticipated, it is even necessary to know something of the hazards of the chemical to domestic animals. If the distributed product is potentially hazardous, safe and adequate directions for use understandable to the potential user must be worked out.

In developing the information for attaining the objectives desired by the three groups, there have been statements, in many ways by many people, to the effect that in the final analysis it is the experience with man that really counts. However, this experience with man may be painful, perhaps even tragic, in its consequences, and to a great extent avoidable. To a large extent, the distress accompanying experience in man can be materially reduced if full advantage is taken of the usefulness of animal toxicity experimentation. The case for the usefulness of animal experimentation has been succinctly stated by Dr. J. H. Burn of Oxford University. He said:

> The only method by which sound conclusions can be reached is the experimental method . . . . Biochemical investigations indicate that, so far as concerns the processes by which food and drink are utilized in the body, the rat, the rabbit, and especially the dog, have much in common with man, and that what is true of these animals is also true of man. The rabbit differs in being a herbiverous animal, living on grass and leaves, but the dog has a digestive system very closely resembling that of man, the points of difference between the two being very few.

Some of the pertinent advantages that derive from animal experimentation are:

(1) Relatively large numbers of animals can be used under conditions in which the majority of variables inherent in biological experimentation can be controlled. Environmental factors, such as temperature, humidity and housing, can be kept constant. A diet designed at will can be held constant. A strain of known genetic background and of a predictable incidence of spontaneous lesions may be chosen.

(2) At any given selected time during animal experiments, the animals can be sacrificed for exhaustive or specific histological examinations. Damage not detected by the usual whole animal organ function tests can often be found by the pathologist.

(3) The life span of most laboratory animals is such that several years of equivalent experience in man can be telescoped into a few weeks or months of animal experimentation, and a whole lifetime into two rat years. Abundant evidence that this can be done exists in the field of chemical carcinogenesis. Here the effects in man of ten to 30 years' exposure to a carcinogen have been duplicated in 12 to 30 months in laboratory animals.

(4) Moral considerations prevent the pushing up of dosages in clinical experimentation. In the experimental laboratory, comparable data can be developed without jeopardy to human life. Doses of a chemical can be pushed to the point of actual damage. Thus, in many cases it is possible to discover in a relatively short time, by larger dosages, an injury that a smaller amount of the chemical may only infrequently produce in man.

(5) In the vast majority of biochemical systems occurring in nature, we find a remarkable similarity, whether the system is in a yeast cell, a mouse liver, or a man. The differences are usually minor and infrequent, and have been overemphasized. It is important, of course, to be aware of the existence of these differences. In fact, when such differences in biochemical systems with species have been found and have become understood, they have become important research tools for the biologist. Most striking evidence for this point is found in the entire field of antibiotic therapy. Here selective toxicity with respect to species differentiation has been exploited with happy results for one of the species—man.

It would be foolhardy to pretend that all possible effects in man can be predicted from results of animal experimentation. Some of the areas that have been troublesome are analgesic, sedative, stimulant or depressant effects on the higher brain centers; blood and bone marrow disturbances; sensitizators; and chemotherapeutic activity.

Man is the only satisfactory test animal for studying the effects on the central nervous system mentioned above. Fortunately, nearly all of the effects are readily reversible, and no great harm results from experimentation begun cautiously. It goes without saying, of course, that any drug to be so tested should have been thoroughly evaluated in animals for other toxic effects. Although it is necessary to use man to evaluate the usefulness of central nervous system drugs—an anesthetic, for example—there are some ways in which animal experi-

mentation can lead to useful information on the effect of a chemical upon the nervous system. Various performance tests have been devised for animals, such as solving mazes; climbing or clinging to vertical mesh devices; behavior following normally provocative stimuli; and electric- or sound-induced behavior, and counteracting effects of known drugs. Generally, wherever activity in this category is found in animals it will be found in man. However, drugs which fail to produce effects in animals may still produce them in man. Finally, quantitative comparisons of the activities of several compounds obtained in animal behavior tests seldom are valid in man.

Bone-marrow disturbances have to date remained virtually unpredictable from animal experimentation. A number of chemicals such as the hydrazines, aromatic amines and aromatic nitro compounds produce clear-cut effects on the formed elements in the blood of animals. Whenever this has occurred, similar or more severe effects have been noted in man. However, the less common drug-induced anemias and granulocytopenias have come to light only after extensive clinical trial. Even in experimentation on man, there is some doubt that such a drug property would be found, since it may occur in less than one in a thousand cases. It is only after the chemical comes into wider use that this potential can be adequately evaluated.

One of the major problems to be faced in the development of products of any kind that will come into contact with man—whether the products are cosmetics, drugs, lubricants, clothing, plastics, adhesives or industrial chemicals—is the chemically induced allergic reaction, usually sensitization of the skin. Many a product otherwise excellent in every respect has fallen by the wayside only because it produced a skin rash or sensitization. Most animal species are not subject to sensitization of the skin. Guinea pig skin will become sensitized to some, but not all, potent sensitizers. Monkey skin can also be sensitized, but practically nothing is known of how this parallels human experience. The remainder of the common laboratory animals are useless for assessing sensitization propensities. This leaves only man. Here, again, the situation is not a happy one. Sensitization, once produced, persists for many years so that a human volunteer, once sensitized, will react to the same chemical should he be again exposed during practically the remainder of his life. Added to this ethical problem is the additional predicament of how nonsensitizing

a product should be. It might, for example, take as many as 300 volunteers to pick up an incidence of 1 per cent sensitization.

The chemotherapeutic activity of a chemical against an infectious disease in man is strictly outside the scope of this discussion. Suffice it to say that animal work in this connection is only as good as the experimental infection.

Good examples of predictability from animals to man in their reaction to chemicals are difficult to point out, for the following reasons: In many cases where good parallelism has been shown to exist, the toxic reaction for one reason or another was seen first in man. Subsequent work showed that under proper conditions similar toxicity could be found in animals. This was true with diethylene glycol, B-naphthylamine bladder tumors, skin cancer in chimney sweeps, damage from radiation, addiction with morphine, toxicity of lithium chloride as a salt substitute, and a host of others. It can be argued and frequently is, that "sure, you found toxicity in animals, because you knew what to look for." Furthermore, when an investigator finds an interesting correlation between experience first seen in man and subsequent laboratory findings, it is publishable. A negative correlation is seldom publishable.

A second area, which is perhaps the larger, where examples might well exist, cannot easily be evaluated in a positive way. This is the area in which a considerable amount of animal work has been done: the safe level or dose estimated from the animal data, and these levels subsequently used in man. So far as we are aware, there have been very few cases where experience in man has contradicted this estimate of safe dosage. This may be the result of good parallelism between man and animals or the result of conservatism in estimating the safe human level. Probably it is the result of both. Unfortunately, this entire area, as long at it operates satisfactorily, achieves no publicity. Yet in the case of chemicals studied and handled in this way, there are probably the best and greatest numbers of parallel effects in man and animals. Practically all of the drugs developed since 1938 fall into this category; so do the synthetic insecticides, pesticides and fungicides developed within the past 20 years, the vast majority of chemical food additives now in common use and the more recently developed potentially hazardous industrial chemicals.

In reviewing the above-mentioned area in which there has been considerable success in evaluating animal toxicity in terms of potential toxicity in man, what have been the most useful guideposts? What factors have influenced the evaluation of animal-toxicity data? What general observations can be made regarding the various phases of animal toxicity work?

Perhaps the major lesson that has been learned from the review of animal data on several thousand compounds is that in order successfully to evaluate potential toxicity in man we must have some understanding of each of the many facets of the action of the chemical. Acute- and chronic-toxicity experiments alone are not enough. We must have data also on histopathology, hematology, pharmacodynamics, irritation and sensitization. Studies on absorption, distribution, excretion and duration of sojourn in the body are also helpful.

We have been impressed again and again with the necessity of using a variety of species of animals for each of the various phases of the study of a chemical. In our own laboratory we have at times been guilty of hindsight thinking in this regard. Although we had made extensive toxicity studies on monochloroacetic acid, it was not until a mysterious outbreak of vomiting and diarrhea in some troops during World War II, following their consumption of an orange drink, that we discovered that the offending agent was added monochloroacetic acid, and that it also caused vomiting and diarrhea in dogs. Later, we had a similar experience with one of the coal-tar colors—FD&C Orange No. 1. In recent years we have become increasingly aware of a number of biochemical differences among the various species. A realization of some of these biochemical differences logically leads to the selection or rejection of a certain species for a particular experiment. Dogs, for example, have a poor acetylation mechanism for aromatic amines. On the other hand, dogs or cats are the species of choice for the study of compounds producing methemoglobinemia. There are numerous other examples of this type.

Several investigators have reported on the value of relative organ weights in revealing potential toxic effects.

Another observation has been that great advantage accrues to those laboratories in which the various investigators, biochemists, physiologists, pharmacologists and pathologists work together in a close-knit or integrated group. In such an organization, the various

investigators can be in constant touch with the various phases of study of a particular chemical. The pathologist, for example, can participate in the planning and conduct of the experiment, as well as be present during the autopsy of chronic animals. Too often the only contact the pathologist has had with an experiment is the study of little blocks of tissue furnished by someone else not a pathologist.

Finally, when the pharmacologists have completed their animal studies and the biochemists, physiologists and pathologists have made their associated investigations, they will have accumulated a mass of quantitative data and qualitative observations which then must be used to arrive at some dosage level, tolerance or expression with respect to a recommendation for man. A great deal of effort has been expended to work out formulas, therapeutic ratios, margins of safety, toxicity ratios, and the like. On the basis of body weight, most animals are said to be from five to ten times more resistant to a toxic agent than is man, for example. All of these guides are useful, particularly as a starting point in evaluation. However, they should be used as general guides only, since each chemical involves a host of considerations peculiar to this one chemical. There can be no formula, ratio or substitute for the mature and considered judgment of men fitted by experience and training for making the final recommendation. In the last analysis, the caliber of the men conducting the experiments and making the evaluations determines the usefulness of animal-toxicity data. **[The End]**

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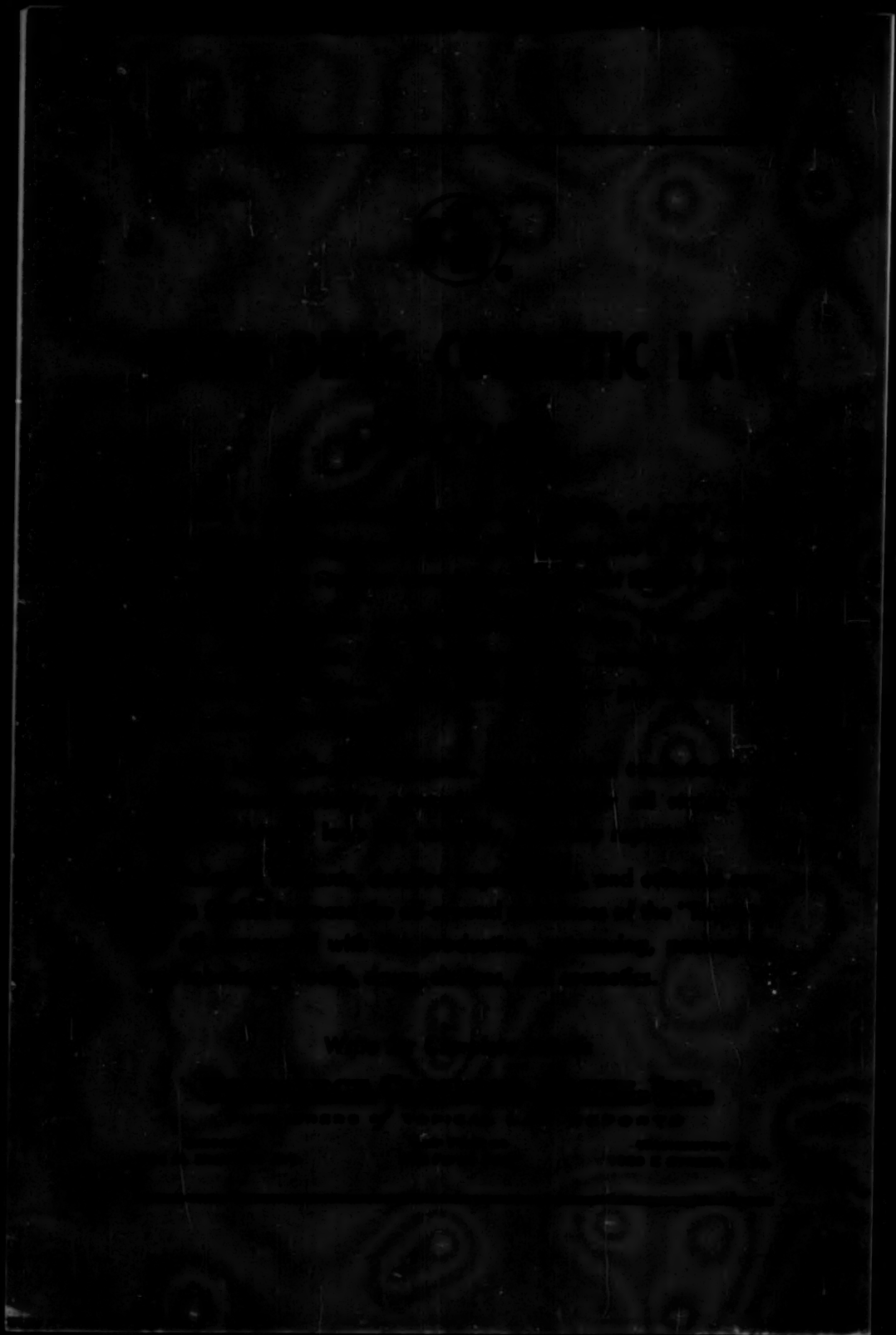
In her present position as assistant in the Chronic Toxicity Branch, **Anne R. Bourke** is devoting her major efforts to a study of chemical carcinogenicity. Before coming to FDA, Dr. Bourke was engaged in immunological studies at the Army Medical School, and also served as bacteriologist with the United States Navy.

John H. Draize entered government service in 1935 as a toxicologist at Edgewood Arsenal. He transferred to the Administration in 1939, where he is now Chief of the Skin Toxicity Branch of the Division of Pharmacology.

Arthur A. Nelson, M. D., spent 22 months as a medical pathologist for the Public Health Service. In November, 1939, he joined the Division of Pharmacology as pathologist, and has served in that capacity ever since.

Bert J. Vos, M. D., came to FDA in the same year. He is now Assistant Chief of the Division of Pharmacology. His main interests are biological assay and experiment design and interpretation.

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